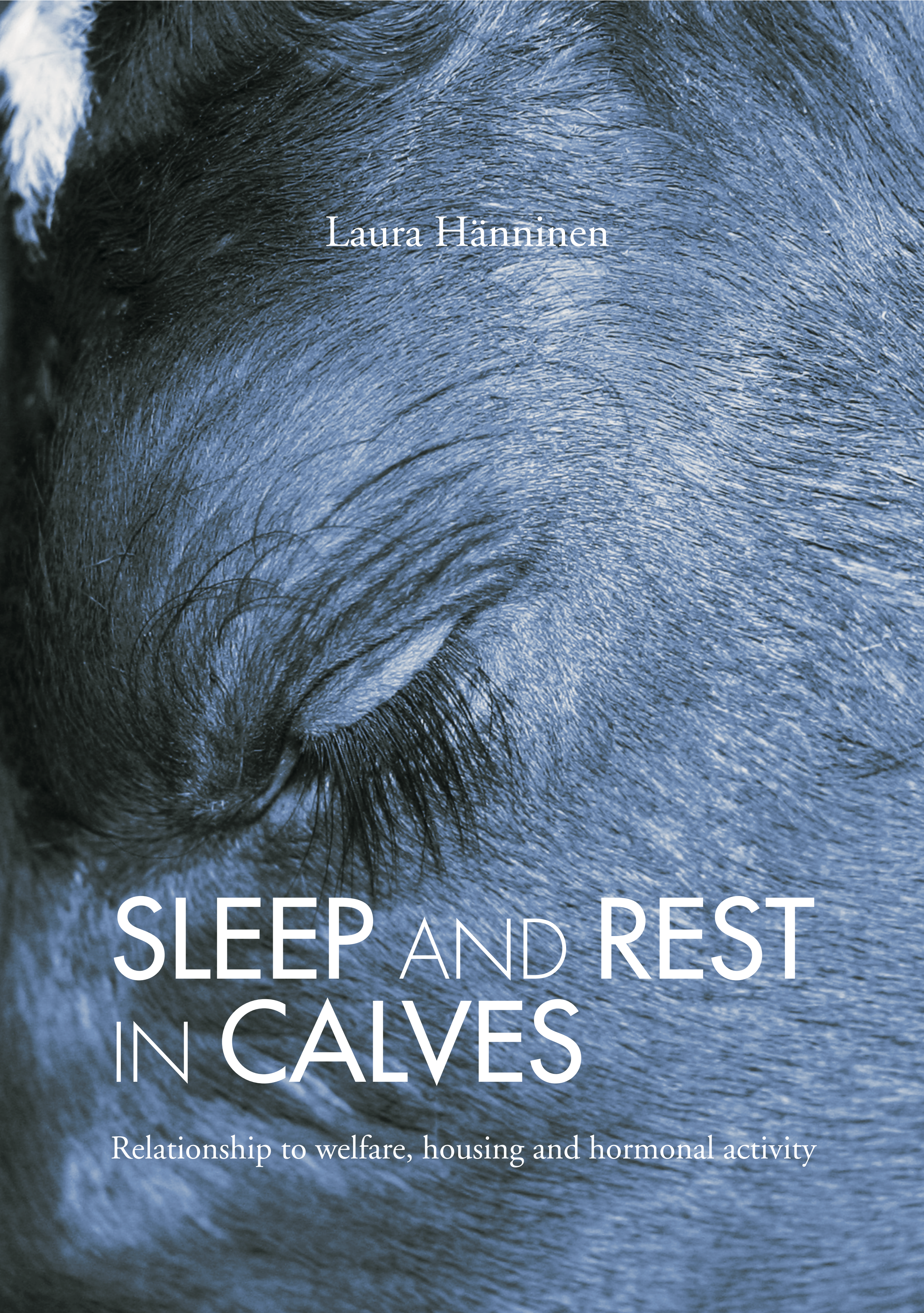

Laura Hänninen

SLEEP AND REST IN CALVES

Relationship to welfare, housing and hormonal activity

SLEEP AND REST IN CALVES
RELATIONSHIP TO WELFARE, HOUSING AND HORMONAL ACTIVITY

ACADEMIC DISSERTATION

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To my first supervisor
Heli Castren, DVM, PhD
(1955 – 1996)

”Ja kiltit kailavat lehmien lapset
nukkuvat nurmella,
ja yökkönen kehrää ja kuusta tuulee,
nukkuu vasikka.
Ja apilamyssynsä alta sen kuulee
nukkuva vasikka.”

KAARINA HELAKISA
LEHMÄSERENADI MAKEA KUIN MARMELADI
(A COW SERENADE SWEET AS MARMALADE)

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ABSTRACT

Adequate rest and sleep is essential for the welfare of young growing animals. Sleep regulates the secretion of several hormones, such as growth-hormone (GH) and glucocorticoids, and is essential for brain development. Sleep has yet not been shown to be related to GH and cortisol secretion in cattle, but lying deprivation affects the pituitary-adrenal axis in bulls and reduces GH secretion in dairy cows. Several environmental factors are thought to affect calves' sleep and rest. The effects of housing or management on the sleep and rest of farm animals is therefore of concern regarding the animals' welfare. The rhythms of rest and activity of calves have been proposed as potential measures of the extent to which farm animals have adapted to their environment.

Thus far, however, very little systematic work has been conducted in order to identify and understand the meaning of resting behavior for calves. What is the relationship between resting and sleep in calves? How possible stress caused by hard flooring or relocation would affect the secretion of rest-related hormones such as GH and cortisol in calves. Which environmental factors may influence their rest at different ages, and how?

In this dissertation, a non-invasive method to register sleep electrophysiologically from young calves was developed, and the electrophysiological findings were validated against their resting behavior to find the best behavioral estimates for identifying sleep phases. Also, the questions raised above were studied by following calves' resting behavior in different rearing environments; calves were followed for three months during different seasons in cold and warm group housing systems, and their behavior and performance was compared to those of calves kept in more traditional, individual pens. In addition, as soft bedding is an important welfare-factor for adult cows, calves' resting behavior was recorded on softer and harder floorings over a five-month period. To deepen our knowledge of the relationship between resting behavior and rest-related endocrinology, calves' GH and cortisol secretion patterns were followed after relocation or housing on softer or harder floor types.

Electrophysiological data can be recorded non-invasively from freely moving, group-housed calves. Three-month old calves slept for 25 % of all observations in 47 bouts of 5 min per day. Rapid eye movement (REM) sleep comprised 45 % and non-REM (NREM) sleep 55 % of the calves' total sleep time. Sleeping-behaviours are a good measures for the total daily sleeping time and the overall time spent of NREM and REM sleep daily, but poorer for

differentiating the NREM and REM rhythm or the bout length over short observation epochs. It is possible to identify calves' sleep if they are resting with their head still or on the flank: the occurrence of these behaviors accurately identifies 78% of the time when the calf was asleep Observations of the calf resting quiet with its head up predicted only 55 % of the 30s epochs of NREM sleep. The best behavioural predictor of REM sleep was the calf resting with neck relaxed, which predicted 61 % of the 30s epochs of REM sleep.

Calves' total resting time and daily resting rhythm was affected very little. As the calves grew older, the daily time they spent resting remained constant, above 50-60% of the day, but the proportions of time they spent resting on the side decreased. In addition, one-to-two-week old calves seldom rest on their sides. This was probably due to an energy saving strategy, as low temperatures can directly affect the resting behavior of the un-weaned calves during the first months of their lives, thus increasing their total resting time and decreasing the time spent resting on their sides. Their circadian resting rhythms were only slightly affected by the weaning; the time spent resting around feeding times decreased after the calves' were weaned off milk. Pair-housed calves were able to use the larger pen area and rested more often and for longer durations on their sides than did the individually-housed calves.

The softness of the floor had little effect on the growth or behavior of the calves, although the bedding used may have alleviated the hardness of concrete floor. Flooring or relocation had no overall effect on the number of pulses, mean GH or cortisol concentrations. However, the effect of time of the day depended on the floor type; calves on concrete floors had higher cortisol concentrations than did those on rubber mats, especially during the night. Relocation had no effect on mean GH or cortisol concentrations and only a small effect on the nature of the pulse variables and the ultradian GH concentration rhythm in plasma. Pulse intervals for cortisol tended to be longer for relocated calves, but the mean cortisol concentrations immediately after the relocation were substantially less than the usual cortisol concentration following acute stress.

In conclusion, electrophysiological data can be recorded non-invasively from freely moving, group-housed calves, and observations of resting behavior can identify when calves are asleep, although further work is needed to use behavior to identify the phases of sleep. The calves effectively maintain their total resting time under several potential environmental stressors such as low temperature, isolation, and hard flooring. Aging and environmental conditions mainly affect the proportions of different resting postures rather than the total time spent resting. As with the resting behavior, stressors such as relocation or floor type do not easily affect the levels of rest-related hormones such as cortisol and GH. Consequently, resting time may not be a sufficiently sensitive measure of environmental quality in young calves, unless the environment is very stressful. To identify those environmental factors which may disturb calves' sleep will require additional research.

LIST OF ORIGINAL ARTICLES

This thesis is based on the following original articles, referred to in the text by their Roman numerals:

I Hänninen L., Hepola H., Rushen J., de Passillé A. M., Pursiainen P., Tuure V.-M., Syrjälä-Qvist L., Pyykkönen M., Saloniemi H. Resting behavior, growth and diarrhoea incidence rate of young dairy calves housed individually or in groups in warm or cold buildings. *Acta Agriculturae Scandinavica Section A-Animal Science* 2003; 53: 21-28.

II Hänninen L., de Passillé A. M., Rushen J. The effect of flooring type and social grouping on the rest and growth of dairy calves. *Applied Animal Behavior Science* 2005; 91: 193-204.

III Hänninen L., Lœvendahl, P., de Passillé A. M., Rushen J. The effect of floor type or relocation on calves' pulsatile growth hormone and cortisol secretion, *Acta Agriculturae Scandinavica Section A-Animal Science* 2006; 56:99 – 108.

IV Hänninen L., Mäkelä, J., de Passillé A. M., Rushen J., Saloniemi, H. Assessing vigilance state in pair-housed calves through electrophysiological and behavioural recordings - a preliminary study, *Applied Animal Behavior Science* 2006, submitted.

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I INTRODUCTION

Adequate rest and sleep is essential for the welfare of young growing animals (*Rechtschaffen* 1998; *Everson* 1995; *Siegel* 2005). Sleep regulates the secretion of several hormones, such as GH and glucocorticoids (*Steiger* 2002) and is essential for brain development (*Mirmiran* 1986; *Morrissey et al.* 2004; *Siegel* 2005). Therefore the effects of housing or management on the sleep of farm animals are of concern with regard to the animals' welfare. Several environmental factors, are thought to affect calves' sleep (*Ruckebush* 1972; *de Wilt* 1985; *Galland et al.* 1993) and rest (*de Wilt* 1985; *Bøe & Havrevoll* 1993; *Sato & Kuroda* 1993; *Bokkers & Koene* 2001), and a positive correlation between the amount of rest and growth rates has been reported for growing cattle (*Mogensen et al.* 1997).

Hitherto, very little systematic work has been conducted to define, identify, and understand the meaning of resting behavior for calves. Why do the calves rest as much as they do, and which environmental factors may affect their rest at different ages, and how? What is the relationship between resting and sleep in calves? We also lack a validated, non-invasive method for recording sleep states in group-housed, freely-moving cattle. And further, because animal species have their typical circadian behavioral and endocrinological rhythms, which can be disturbed by stressors, it is also important to emphasize daily patterns when studying resting behavior.

Welfare is defined in this thesis as calves' capacity to cope in the environment (*Broom* 1988). Several authors have suggested that sleep patterns (*Ruckebush* 1975) or activity rhythms (*Ruckebush* 1975; *Veissier et al.* 1989; *Scheibe et al.* 1999) could serve as a measure for the adaptation of animals to their environment. Changes in the frequency or duration of sleep or rest episodes may reflect the degree to which animals have adapted to changes in their physical or social environment or diet (*Ruckebush* 1975). However, to use resting behavior as a tool for judging calves environment, we must define, identify, and understand the behavior. In the literature review, I discuss some of the environmental factors affecting calves' resting behavior and welfare.

In this thesis I studied systemically and experimentally the calves' rearing environment. This dissertation focuses primarily on the effect of environmental comfort (temperature, floor quality, and social company) on calves' resting behavior (studies I-II). To deepen our knowledge of the relationship between resting behavior and rest-related endocrinology, we experimentally manipulated the calves' environment by relocating them and keeping them on different floor types in order to identify changes in GH and cortisol secretion patterns (study III). In addition, as sleep is a fundamental though vaguely studied part of the resting behavior in farm animals, we developed a non-invasive method to register EEG from young calves. We validated the EEG findings against the calves' resting behavior to identify their resting postures, and thus to determine the best behavioral estimates for their sleep phases (study IV).

II LITERATURE REVIEW

Several internal and external factors may affect the resting behavior of calves, some of which appear in Table 1. However, this literature review focuses only on the most pertinent results concerning the factors studied in this thesis. Because this thesis is focuses on young calves, the results from the studies obtained from adult cows are discussed only, if particularly relevant.

1. CALVES' RESTING BEHAVIOR

Most newborn calves are hiders: the dam hides the calf away from the main herd during the first 3-4 days to ensure the bonding of the calf to the dam. During that period, the calves mainly lie down except during 4-6 nursing periods (*Lidfors* 1994). When introduced to the main herd, the calves tend to prefer certain resting areas where they return to lie down near preferable rest mates (*Bouissou & Hövels* 1976; *Reinhardt et al.* 1978; *Bouissou et al.* 2001).

Five to six month old calves are shown to rest over 50% their day in semi-wild environment (see. (*Le-Neindre* 1993) and 60-80% in barns (*de Wilt* 1985; *Ketelaar-de et al.* 1990; *Albright et al.* 1991; *Le-Neindre* 1993; *Panivivat et al.* 2004). The overall time spent resting is decreasing only slightly as the calves grew older (*Bøe & Havrevoll* 1993; *Sato & Kuroda* 1993; *Panivivat et al.* 2004). Calves spent only 1-2% of the day resting on their sides (*Albright et al.* 1991; *Le-Neindre* 1993). The function of different resting postures remains unclear; it may involve temperature regulation and reflect sleep patterns.

Table 1. A selection of publications reporting some factors affecting the calves' resting behavior on- farms

Environmental temperature
Gonzalez-Jimenez & Blaxter 1962
Brunsvold et al. 1985
Bøe & Havrevoll 1993
Schrama et al. 1993
Schrama et al. 1995
Kauppinen 2000

Space allowance
de Wilt 1985
Ketelaar-de et al. 1990
Le-Neindre 1993
Wilson et al. 1999
TapkI et al. 2006

Social company
Warnick et al. 1977
de Wilt 1985
Ketelaar-de et al. 1990
Albright et al. 1991
Le-Neindre 1993
Chua et al. 2002
Babu et al. 2004

Lightning
Weiguo & Phillips 1991

Age
de Wilt 1985
Ketelaar-de et al. 1990
Le-Neindre 1993
Albright et al. 1991
Bøe & Havrevoll 1993
Sato & Kuroda 1993
Panivivat et al. 2004

Feeding
McFarlane et al. 1988
Broucek et al. 1992
Sato & Kuroda 1993
Bokkers & Koene 2001
Phillips 2004

Environmental changes
Veissier et al. 1989
Johannesson & Ladewig 2000

Softness of the lying surface
Wilson et al. 1998
Stefanowska et al. 2002 S
Panivivat et al. 2004

Weaning
Veissier et al. 1989

Tail docking and/or dehorning
Tom et al. 2002

2. SLEEP

Zepelin et al. (2005) defines mammalian sleep as an individual that sustains *"quiescence in a species-specific posture accompanied by reduced responsiveness to external stimuli, has a quick reversibility to the wakeful condition and characteristic changes in the electroencephalogram"*. Although few exceptions from the previous definition have been found in animals, thus far the sleep of all our mammalian farm animals fits this definition.

2.1 REGISTERING SLEEP

Sleep is commonly measured by registering both sleeping behavior and brain electrophysiology, EEG (electroencephalogram). Sleep states in animals can be identified through the animal's behavior, which has been successfully demonstrated in herbivores in zoos (*Tobler* 1992; *Tobler & Schwierin* 1996), and in laboratory animal species, such as macaques (*Balzamo et al.* 1998) and mice (*Storch et al.* 2004). In REM sleep, neck muscles are atonic such that the neck cannot support the head, and eyes move rapidly and sporadically both horizontally and vertically (i.e. rapid eye movement sleep). Thus, as Ruckebush (1965; 1972; 1974b) and de Wilt (1985) have suggested, sleep states in cattle can be identified through observations of resting body postures and eye activity. However, we need more information on how strongly these behaviors correlate with sleep states in different species.

Few studies on farm animal sleep have been conducted during the past 15 years. In these studies, electrodes have been either implanted surgically trough the skull (*Ruckebusch et al.* 1974; *Robert & Dallaire* 1986; *Grant et al.* 1995) or subcutaneously with needles (*Lewin & Tonhardt* 1998; *Takeuchi et al.* 1998). In these studies, the horses and cattle were isolated, tethered, or restrained. Ambulatory EEG registering techniques using subcutaneous needle electrodes in horses (*Giovagnoli et al.* 1996; *Lewin & Tonhardt* 1998) and zinc-cup electrodes in sheep (*Langford et al.* 2002) have been successful in recent tests.

2.2 THE STRUCTURE OF SLEEP

Electrophysiologically sleep is split into two main phases: rapid eye movement sleep (REM), also called a paradoxical sleep, or active sleep and non-rapid eye movement sleep (NREM), also called quiet sleep, orthodoxical sleep, or slow wave sleep (SWS). Occasionally, terms can seem contradictory. For example, in humans and in some laboratory animal species, NREM has been divided into four phases of sleep depth. In this case, only the deepest phases (3 and 4) of the NREM sleep, are called slow wave sleep (*Tobler* 1995). In many species, however, scientists use the term SWS to describe all NREM sleep, which is interrupted by shorter REM phases (*Elgar et al.* 1988; *Tobler* 1995). In this thesis, I chose to use REM and NREM, to describe the two main phases of sleep.

In principal, the smaller the animal and the northern it lives, the shorter the daily sleeping duration and the shorter the REM-phases. The stimulus threshold to wake up from REM-sleep is higher than that of NREM, and thus a long REM-sleep period can be a threat to survival for prey species other than those rodents species that sleep in nests (*Allison & Cicchetti* 1976; *Elgar et al.* 1988; *Tobler* 1995). Primates often exhibit a mono- or biphasic sleeping rhythm while several other species have a polyphasic rhythm, sleeping all over the day. Typical examples of polyphasic short sleepers are our domestic ruminant species, such as cattle, sheep, and goats.

The sleep cycle consists of one or several REM and NREM phases. The cycle length is species-specific. Sleep cycle lengths are short in farm animals, as in several prey animal species. Total daily sleeping duration also varies between species. These differences between species depend on several factors, such as time spent eating, and available food sources, digestion and rumination, and ecological niche (*Allison & Cicchetti* 1976; *Elgar et al.* 1988; *Tobler* 1995). Grazing animals, for example, are assumed to sleep less, as they need more time to consume large amounts of low- calorie foods (*Siegel* 2005).

In mature mammals and birds, the sleep phase usually starts with NREM and deepens during the REM phase (*Zepelin et al.* 2005). The young of many terrestrial mammalian species sleep more and have more REM sleep than do older animals (*Siegel* 2005). Sleep is essential for brain development, and REM sleep is connected to the early developmental phase (*Mirmiran* 1986; *Morrissey et al.* 2004). Young animals have also higher need for energy conservation acquired through sleep. Precocial young mammals, such as bovine calves, are suggested to spend proportionally less in REM sleep from their total sleep time than do young altricial mammals (*Siegel* 2005).

2.3. SUGGESTED FUNCTIONS OF THE DIFFERENT SLEEP PHASES AND EFFECTS OF DEPRIVATION

Sleep is a vital body function, regulating several biological phenomena. Many theories have been presented to explain the function of sleep and sleep phases. Most theories assume that sleep serves the same functions for all animal species. However, only a few species have been studied until now (*Siegel* 2005).

Deprivation studies are one of the ways used to examine the physiological functions and the regulation of the sleep. Sleep deprivation is a stressor, and its' effects depend on an individual's prior sleep deficit and distribution during the day. Sleep deprivation can be partial, total, acute, or chronic or specifically focused on one of the sleep phases. Sleep deprivation affects a large spectrum of vital systems such as thermoregulation, energy balance, and immunofunction (*Bonnet* 2005). If sleep loss is chronic, depriving individual animal totally from sleep or selectively from REM or NREM sleep, experimental animals will die within a month due to infections or metabolic disorders. Hormones, which are dependent on sleep, loose their secretion rhythm. Several sleep-related hormonal secretions, such as GH and prolactin, diminish, when sleep is deprived. The body temperature decreases before an individual falls asleep, but during sleep deprivation, the body temperature remains around the normal level (*Bonnet* 2005).

During NREM sleep, body metabolism slows, body temperature decreases, and oxygen consumption diminishes, so that energy is conserved (*Shapiro & Flanigan* 1993). Brain glycogen storage is restored during NREM sleep (*Benington & Heller* 1995) and anabolic processes accelerate (*Shapiro & Flanigan* 1993). NREM sleep may also play a central role in neurogenesis; REM sleep occurs proportionally and absolutely more during early development, and secures neuronal development (*Siegel* 2005).

2.5 CATTLE SLEEP

Little is known about sleep in cattle. Ruckebush (1965; 1972; 1974b) did studies only with four cows, all with surgically-implanted electrodes via the skull, and de Wilt (1985) based his suggestions only on behavioral observations. According to Ruckebush (1972) adult cattle sleep about four hours per day, but they compensate by drowsing for twice this amount of time. Cattle naturally sleep while lying down and must lie down to have REM sleep, which typically occurs in short periods of 2-8 min (*Ruckebush* 1972; *Ruckebusch* 1974b). They can, however, have NREM sleep when standing, if forced to (*Ruckebusch* 1974b). Cattle can ruminate during NREM sleep, but not during REM sleep because rumen motility decreases during REM sleep, and gas burping is inhibited (*Itabisashi* 1973; *Ruckebusch et al.* 1974). Itabisashi (1973) suggested that gas in the reticulo-rumen might stimulate the rumen receptors and rupture the REM-sleep phase.

In an isolated and soundproof environment, adult cattle are sleeping polyphasicly throughout the day. Adult cattle in the pasture, however, are mainly crepuscular, being active mostly at dawn or dusk. Inside cow barns, the amount of REM and NREM sleep was double the amount at the pasture.

Also, most of the REM-phases of sleep occurred at night inside the cow barn (*Ruckebusch* 1974a).

3. CIRCADIAN RHYTHMS

Biological rhythms, such as hormonal fluctuation and rest-activity or sleep rhythms, are generated by an internal system. All animals benefit from restricting their activity to times of the day when optimal environmental conditions exist; when it is not too dangerous to eat or sleep. Biological rhythms are classified as circadian, with a cycle of approximately 24-hours, and ultradian, with a cycle less than 24 hours.

The main regulator, pacemaker, is in the suprachiasmatic nuclei (SCN). The SCN is situated in the hypothalamus, just directly above the optic chiasm (*Buijs et al.* 2003). The suprachiasmatic nucleuos functions already in fetuses (*Weinert* 2005), such as in lambs (*Yellon & Longo* 1987). The main regulators for the SCN are external light and feeding. Other factors that synchronize the circadian system are, for example, nutrition, hormone feed-back mechanisms, activity, and social cues (*Buijs et al.* 2003).

4. CORTISOL, GH AND RESTING BEHAVIOR

Cortisol and GH are both circadian hormones; their secretion rhythms are regulated by the SCN (*Giustina & Veldhuis* 1998). The hypothalamus-pituitary-adrenocortical (HPA)-axis is the major endocrinological pathway for reacting in stressful situations. When exposed to stress, CRH from the hypothalamus stimulates pituitary ACTH secretion, which leads to an increased glucocorticoid release from the adrenal glands. The secretion of cortisol, major glucocorticoids secreted in stressful situations, is inhibited by the negative feed-back and ACTH secretion (*Bøe & Havrevoll* 1993; *Sjaastad et al.* 2003).

Growth hormone (GH) secretion from the hypophysis is regulated mainly by the interaction of two hypothalamic hormones; growth hormone releasing hormone (GHRH) and inhibiting somatostatin (SS). Also, SS is known to inhibit GHRH activity, and GH induces SS secretion. Several neuropeptides are also found to be involved in the triggering or inhibiting of GH secretion (*Bluet-Pajot et al.* 1998; *McMahon et al.* 2001; *Sjaastad et al.* 2003). In addition, physiological amounts of glucocorticoids are needed for normal GH production, but controversial results exist between species or stressful stimuli on the reaction of GH secretion. The responses of the GH axis depend on glucocorticoid dosage and timing (*Giustina & Veldhuis* 1998). In cattle, for example, acute thermal exposure increased (*Mitra & Johnson* 1972) and chronic thermal load decreased the GH secretion (*Mitra et al.* 1972).

4.2 Circadian rhythms in behavior and hormone concentrations

Sleep is a physiological regulator for GH and glucocrticoid secretions in laboratory animals and humans (see rev. (*Steiger* 2002) and is associated with GH secretion in sheep (*Laurentie et al.* 1989), but not in goats (*Tindal et al.* 1978). No relation has yet been shown between sleep and GH and cortisol secretion in cattle, but deprivation of lying does affect the pituitary-adrenal axis in bulls (*Munksgaard et al.* 1999) and reduces GH secretion in dairy cows (*Munksgaard & Løvendahl* 1993).

Sleep onset stimulates GH secretion, but the hormones of the somatotrophic axis are also involved in sleep regulation in a complex way. GHRH, for example, stimulates slow wave sleep and slow wave activity, and GH increases REM sleep. The secretion of GH increases during sleep independent of the circadian sleeping cycle, and sleep deprivation diminishes the GH release. In humans, however, a day time GH secretion increases after one night of sleep deprivation, thus partly compensating the loss (*Brandenberger et al.* 2000).

In humans and laboratory animal species studied so far, blood cortisol is the highest at wake onset early in the morning, and is the lowest in the late evening and early part of the sleep period. Thus, sleep is normally set in when corticotropic activity is quiescent. Cortisol begins to rise a few hours before the usual waking time. The cortisol rhythm is very stable, but sleep deprivation changes the pulse amplitudes (*Van Cauter* 2005).

4.2 Measuring pulsatile hormone secretion

Both cortisol and GH are known to have episodic, pulsatile secretion patterns with ultradian variation in adult cattle (*Lefcourt et al.* 1993; *Lefcourt et al.* 1995), and stress can affect the nature of the pulsatile release of cortisol in bulls (*Ladewig & Smidt* 1989). That's why new methods of analyses are needed to replace the traditional approach of focusing only on basal hormonal levels.

There is relatively little data available on the daily pulsatile pattern of cortisol and GH in young calves. Methods for assessing changes in the pulsatile secretion of GH and cortisol are necessary to fully evaluate the possible effects of stressors on the animals' welfare. Flying-mean and area-under-curve are both methods used to analyze differences between fluctuating basal blood hormone concentrations. However, as the blood hormone concentration is a function of synchronized and degraded hormones, the actual pattern should be distinct under considerable noise.

To improve the detection of sustained GH pulses, Woolliams et al. (1993) modified a non-parametrical method previously developed by Breier et al. (1986). Their method describes a pulse as an upward shift in concentration that passes a set threshold value. The threshold is set according to the assay duplicate standard deviation, with a chi-square value giving optional protection. Such a method may also improve the detection of secretory episodes underlying HPA axis activity, and improve our ability to detect more subtle influences of stress on HPA axis activity and GH secretion.

5. BARN ENVIRONMENT AND RESTING BEHAVIOR

5.1 TEMPERATURE REGULATION AND RESTING BEHAVIOR

Keeping cattle in simple unheated buildings is of increasing interest among farmers who seek for lower building costs. Calves may conserve energy when resting; Schrama et al. (1993) showed that body posture affected the lower critical temperature (LCT: the air temperature below which an animal must elevate its heat production), which was +17 C^0 for a 6-day-old standing calf, and +13.5 C^0 for a lying one. Calves can tolerate relatively low temperatures. The younger the calf, the more susceptible it is to cold stress, as young calves have very little body insulation (*Gonzalez-Jimenez & Blaxter* 1962).

That the amount of resting increases with the cooling of the temperature has also been demonstrated in field studies, for example, in steers (*Gonyou et al.* 1979; *Redbo et al.* 1996), cows (*Malechek & Smith* 1976), and calves by Kauppinen (2000), though Bøe and Havrevoll (1993) found no such relationship. Researchers have suggested that adult cattle kept in low temperatures reduce their activity and the area of body surface exposed to the air in order to save heat and energy (*Redbo et al.* 1996). Several studies have shown that calves in a cold environment chose to rest with their legs curled under their bodies to restore energy and reduce the area of body surface (*Gonzalez-Jimenez & Blaxter* 1962; *Brunsvold et al.* 1985; *de Wilt* 1985). However, Bøe & Havrevoll (1993) found no correlation between calves' resting postures and a low temperature.

On the other hand, recent studies have shown also a second energy saving strategy in cattle in the extreme low temperature; Instead of lying down and minimizing their expended energy, cattle may increase their standing in order to gain the maximum heat from solar radiation (*Olson & Wallander* 2002).

Calves may also huddle together with a conspecific to seek protection against the cold, as has been observed, for example, in steers (*Redbo et al.* 1996). Calves, too, can huddle together in low temperatures (*Bøe & Havrevoll* 1993; *Kauppinen* 2000), and are also capable of seeking out a comfortable microclimate in cold temperatures (*Brunsvold et al.* 1985).

5.2 Softness of flooring and resting behavior

As bedding material is costly and requires more labour, young calves are usually housed on slatted floors or on solid concrete floors, which may be too hard and cold for calves. The floor type can significantly affect the thermal comfort of calves due to increase or decrease the heat flow from animals, thus having an effect on the LCT (*Bruce* 1979)

Adult dairy cows show a clear preference for soft floors in their stalls (*Manninen et al.* 2002; *Tucker et al.* 2003). Previous studies have shown that for the adult cows, environmental comfort, mainly the softness of flooring, has decreased the number of resting bouts and total daily resting time (*Haley et al.* 2001). Only couple of studies have compared the resting behaviour of calves housed on different beddings and with controversial results; Stefanowska, J et al. (2002) showed, that group-housed dairy calves preferred to rest on wooden slatted floors than on plastic or concrete slats. On the other hand, Panivivat et al. (2004) found no differences in the individually-housed calves' resting behaviour on five different bedding materials (river sand, granite fines, rice hulls, long wheat straw, or soft wood shavings). However, they did find that very young calves had more diarrhoea on sand and granite. The meaning of a soft floor to light-weight calves remains unknown.

5.3 Social company and resting behavior

Cattle are gregarious animals, and calves especially tended to rest in pairs or in small groups at pasture, in preferred locations (*Bouissou & Hövels* 1976; *Bouissou et al.* 2001). Housing young calves in pairs or groups rather than individually has been shown to either decrease the total resting time, especially if the space was restricted (*de Wilt* 1985; *Le-Neindre* 1993), or to have no effect on calves' resting time (*Warnick et al.* 1977; *Albright et al.* 1991; *Chua et al.* 2002).

However, most of the results from previous studies cannot be applied due to current EU space allowances. For example, according to a current EU law, calves are not allowed to be tethered before 6 months of age vs. (*de Wilt* 1985; *Le-Neindre* 1993). And further, calves should be kept in groups from the age of 8 weeks on vs. (*Warnick et al.* 1977; *de Wilt* 1985; *Albright et al.* 1991; *Le-Neindre* 1993).

Thus, total resting time, per se, may not reveal calves' motivation for increased or decreased resting time. Specifically, we lack normal reference values for calves' natural resting body postures at different ages and under various feeding regimes and environmental conditions.

III THE AIMS OF THE STUDY

The aim was to *describe* and *define* calves' resting behavior at different ages and to *identify* behavioral correlates to sleep, as well to study some of the environmental factors that may affect calves' resting behavior. In addition, as GH and cortisol secretions are sleep-related, we studied their circadian secretions under rest-related stress situation.

THE MAIN QUESTIONS WERE:

1. How well can we *identify* calves' sleep from their resting behavior (IV)?
2. How do we *describe* and *define* calves' resting behaviour at different ages (I, II)?
3. How and why does the resting behavior change in different environments (I, II)?
4. How will stress affect the secretion of rest-related hormones such as GH and cortisol (III)?

THE METHODS USED TO FIND AN ANSWER WERE:

1. to develop a non-invasive EEG method and to investigate the correlation of certain resting body postures on the occurrence of vigilance states (IV);
2. to investigate how much do calves rest, and in which postures at 1-3 months (I), and 1-5 months of age (II);
3. to study how would fluctuating temperature (I), social company (I, II) or floor type (II) affect calves resting behaviour;
4. to investigate calves' 24-hour pulsatile GH and cortisol secretion rhythm before and after relocation, kept on harder and softer floors (III)

IV ANIMALS AND RESEARCH METHODS

This thesis consists of four parts based on three experiments (I-IV). We conducted studies both in field conditions (I) where calves' resting behavior and performance was observed during different seasons and years, and in defined laboratory experiments, under more strictly-regulated environmental conditions (II-IV). Part I is a field study in which we studied the effect of temperature. Parts II and III based on one experiment in which we concentrate on the effects of floor type or relocation stress or both on resting behavior (II) and GH and cortisol secretion patterns (III). We took into account the effect of aging and social company on calves' resting behavior in studies I and II. Study IV described the development of non-invasive EEG registering method and focuses on the sleep related behavior and EEG findings.

Calves' feeding and management routines varied between studies and I described them in details in the papers concern. In this section, I provide a summary of the main methods of the data collection. For more detailed description, the reader may refer to the original papers included at the end of the thesis.

The experimental procedures were approved by the animal care committee of the University of Helsinki (I and IV), which is monitored by the district government of Southern Finland or the animal care committee of the Lennoxville Research Centre, which is monitored by the Canadian Council for Animal Care (II and III).

1. ANIMALS

In all of the studies, we used Holstein-Friesian male calves. For studies I and IV, we also selected Ayrshire calves. For studies I and II, we began the experiments on the calves' at the age of one week, and followed their behavior for 8-12 weeks and 20 weeks, respectively. In addition, we tested the EEG registering method (IV), and collected basic data for the circadian GH and cortisol rhythms (III), and for the daily sleeping time and patterns (IV) of 11-12 week-old calves.

2. HOUSING TREATMENTS (I-IV)

In experiment I, we housed calves either individually (INDIV) or in groups of four in one of three group-housing systems; one indoor (INGROUP) and two outdoors (OUTCOLD or OUTWARM). The three group-housing systems were identical in structure. They comprised a straw-bedded shelter and a bark-bedded yard area (see Figures 1 and 2). The temperature range during the experiment is shown in a table 2. The individual pens were in the same cow barn as the INGROUP pens. The 1.0 x 1.2 m -pens had wooden-slatted floors. The calves could have some body contact with the neighbouring calf above the solid plywood walls.

In experiments II and III, we housed calves for 20 weeks (from May 28 to Oct 14) in one of the three housing treatments: 24 calves were housed in pairs in concrete-floor double pens (2.1 m x 1.8 m) (PAIR), 12 calves were individually housed in concrete-floor pens (1.05 m x 1.8 m) and 12 calves were housed individually in the same size pens, but with soft rubber mats (Cloud 9, NRI Industries, Toronto, ON). All the pens were bedded daily with (2 kg) of wood shavings. All the calves could see through the metal bar walls separating the pens, so individually-housed calves had visual, auditory, olfactory, and some tactile contact with neighbouring calves. Rooms were ventilated with electric fans and the mean room temperature during the experiments II was +19 °C (range, +11 to +33 °C) and the experiment III 22 °C (ranges 16 – 26 °C).

In the last experiment (IV), we housed calves in pairs in 2.5 m x 2.8 m straw-bedded solid floor pens. The pens were separated with solid side walls 2.8 m high and an open metal bar front. The pens were placed against the room wall. The solid floors were covered with clinker tiles and the walls with ceramic tiles. Each of the pens had a hayrack, a water bowl, and a metal trough for concentrates during the concentrate feeding times.

Figure 1. An illustration of the outside group pen structures for two groups of four calves (see diagram at the next page).

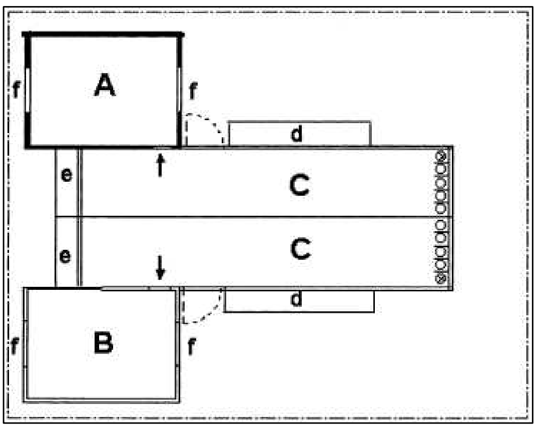
Table 2. Daily mean outside temperatures and daily mean minimum and maximum outside temperatures during the experiment I during the experimental years 1996-1999.

	Daily temperatures (°C)		
Replicate (A-F)	**Mean**	**Min.**	**Max.**
A	-5	-11	+1
B	+4	-5	+13
C	+1	-5	+7
D	-4	-10	-1
E	+7	-6	+16
F	-4	-15	+1

Replicate: two parallel group-housing systems of four calves run outside, one with the heated (OUTWARM) and the other with the unheated (OUTCOLD) shelter.

The outside temperature and the temperature inside the shelters at three heights (20 cm, 80 cm and 200 cm) were recorded four times per hour throughout the experiment with an automatic system built by the Department of Agricultural Engineering and Household Technology, University of Helsinki.

Figure 2. Diagram of the outside group pen structures for two groups of four calves.



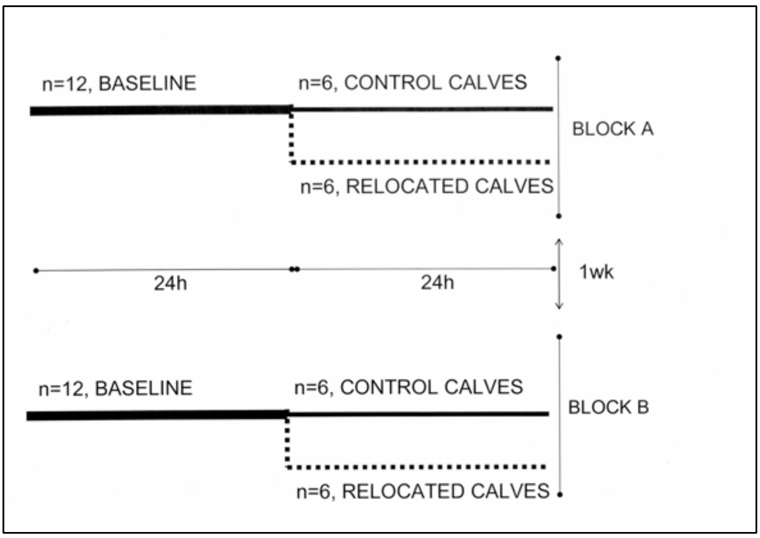
A: OUTWARM: 3×4 m straw-bedded, heated shelter, daily temperature (mean ± SD) +11 ± 6 °C; B: OUTCOLD: 3×4 m straw-bedded unheated shelter, windproof, daily temperature +3 ± 2°C higher than the yard outside; C=2×10m bark-bedded, roof-covered outside yard; d: hay trough; e: concentrate trough; f: window; open circles; teat bucket; crossed circles; heated water bowl (unheated for INGROUP); arrow; plastic strip door; -- --: roof-covered area. The INGROUP housing system was identical in structure to the other two systems.

3. RELOCATION (III)

The calves were randomly assigned to two replicates of 12 calves each (A and B) (Figure 3). The calves of replicate A (6 relocated and 6 control calves) were tested during one week and the calves of replicate B (6 relocated and 6 control calves) were tested during the following week. We used two experiment rooms. Both of the rooms have 24 animals. Of the 24 animals in each room, 6 calves belonged to the first replicate (A) and 6 to the second replicate (B) one week prior. Twelve other animals were housed in pairs and were not participating in this blood sampling experiment.

The experiment calves were relocated to a new room gently, without yelling or hitting. During relocation, one calf from one room changed pens with one calf from the other room. The relocated calves then had one familiar calf as a neighbour in the new room. Two persons guided the calves individually to a cleaned, washed, and freshly-bedded pen. The relocation of six calves took approximately 35 min.

Figure 3. A schematic presentation of the experimental design for the blood sampling and relocation in the experiment III



4. Blood sampling (III)

One day prior to blood sampling, we fitted jugular catheters for frequent blood sampling. We restrained calves with halter, and inserted 18 - gauge needle into *v. jugularis*. We threaded approximately 50 cm of a vinyl tube through the needle, and another 50 cm remained externally. We took the needle away and fitted a three-way valve into the external end of the vinyl tube, and attached the catheter with stitches and covered with elastic linen bandages around the neck. We kept the catheters functional between bleeds by filling them with heparinized saline. Prior to collecting a blood sample, the first 2 ml we drew up was discarded to prevent contamination of the sample with saline. We took the samples (app. 5 ml) with a 5 ml syringe and immediately emptied them into plain glass tubes. Following centrifugation and separation, we stored samples at –80 C^0 until analyzed.

We took blood samples every 20 min throughout the two consecutive 24 h sampling periods (over a 24 h period before the relocation and over a 24 h period following the relocation) (Figure 3).

5. Hormone assays (III)

We determined plasma GH concentrations in duplicate by double antibody RIA (*Peticlerc et al.* 1987). The lower limit of the GH assay was 1.0 ng/ml. The inter- and intra-assay coefficients of variations were 9% and 2%, respectively. All the samples studied had GH concentrations above the detection limit.

We analyzed the cortisol in duplicates with a commercial Elisa kit (No. #402210, NEOGEN corp.). The lower limit of the cortisol analysis was 1 ng/ml. The inter- and intra-assay coefficients of variation were 12 % and 5%, respectively. The usual cortisol concentration following acute stress caused by handling (11 – 20 ng/ml), castration (35 – 45 ng/ml; or dehorning (20 - 35 ng/ml) are clearly above the detection limit (*Lay, Jr. et al.* 1992; *Wohlt et al.* 1994; *Fisher et al.* 1996). 17% from all the cortisol samples had cortisol concentrations below the detection limit. Each of the calf had cortisol samples of which concentrations were less than 1 ng/ml, and these low-concentration samples were distributed around the 24 h.

6. HORMONE PULSE DETECTION (III)

We detected the pulses with the method of Woolliams et al. (1993). A new pulse can occur if there has been a low concentration, (a "trough") since a pulse was last detected. For each of the detected pulses, we defined a number of variables: 1) its time of occurrence, 2) the starting point of the pulse (trough), 3) the mean of the two highest peaks in the pulse (amplitude), 4) and the time between the two consecutive pulses (pulse interval).

7. BEHAVIOR OBSERVATIONS (I,I,IV)

In experiment I, we observed calves' behaviours with interval sampling, and thus behaviors appear as a percentage of total observations. In the experiments II and IV, we used a continuous behavior observation method, and thus measured total time, frequencies, and bout durations. Except for the first replicates of study I, we used Observer software (©Noldus, Netherlands) for the recording and elementary handling of the behavior data. The definitions of the most relevant behaviors recorded during this thesis appear in Table 3.

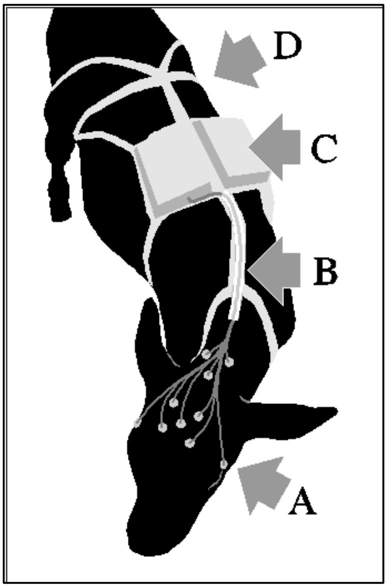
Table 3. Definitions for the behaviors registered in this thesis.

Main resting behaviors	Definitions
Resting on brisket	The calf was resting on sternum
Resting on side	The side of the trunk of the calf was resting on ground.
Total resting	Total time the calf was lying down (sum of the above behaviors)
Resting with head lifted	The calf was lying down with head lifted up, supported by the neck
Resting with head resting against flank	The calf was lying down with head resting against its flank or other upper body part; the neck is not supporting the head
Resting with head resting on the ground	The calf was lying down with head resting against the floor; neck is not supporting the head
Resting with neck relaxed	All neck postures, when the neck is not supporting the head

8. EEG (IV)

8.1 TECHNIQUE

We developed a special non-invasive technique for measuring the sleep states of freely-moving and pair-housed calves without disturbing them (IV). We filled two canvas bags (C), hanging behind the scapulae from the specially constructed harness (D), with two counter weights weighing 400 g each 3 d prior to the registering in order to simulate the experimental situation. At the same time, we covered the calf's head and neck with a soft rubber net and attached it with a rubber band to the halter and harness.



Twenty-four hours prior to the experiment, we sedated each calf (xylatzine 0.06-0.1 mg/kg iv, Rompun® Vet 20 mg/ml, Bayer, Germany) in order to attach the electrodes. We secured altogether, 10 adhesive electrodes (AquaBond, Medibond ltd., Tel Aviv, Israel) (A) to the head and neck according to suggestions described in (*Takeuchi et al.* 1998); monopolaric cephalic electrodes over right frontal, left frontal, right occipital, left occipital and vertex regions, and the ground electrode behind the *os frontalis*. We recorded electromyography (EMG) with two electrodes placed symmetrically on calf's neck muscles and electro-oculography (EOG) with two electrodes placed near the eyes. We connected electrodes to snap-on electrode leads (Nicolet, Nicolet Biomedical inc., Madison, USA), and the leads to a connection wire, which we placed inside a fabric tube (B) along the neck line to the canvas bags (C) hanging on the calf's back.

After we had finished putting the electrodes on, we gave each animal 24 hours rest and recovery time before the recording began. When we began the recording, we attached the calf shortly to the hay rack, and checked the electrical signal quality on the monitor of the portable computer equipped with the appropriate software (Somnologica®, Flaga, Iceland). We replaced one counter weight with an ambulatory polygraphic device (Embla®, Flaga, Iceland.) where electric signals were stored in digital format onto a hard disc. We used the recording bandwidth of 0.05 – 70 Hz for EEG and EOG, and 0.5 – 200 Hz for EMG. The bandwidths were selected to collect the signal frequencies pertinent to sleep scoring. The data was digitized at 300 Hz and stored on a hard disc.

During recording, the animals were loose in their home pens and were accompanied by their pen mates. All the calves were recorded in two sets from 900 to 1430 and from 1630 to 0700; routine barn work was performed during the excluded hours.

8.2 EEG ANALYSES

From each of the two recording sessions per experimental animal, we selected one good-quality trace of electroencephalography EEG, EMG and EOG for standard visual analysis. Using the Somnologica software, we analysed the digital signal in 30 s epochs, at a mean speed of 1 cm/s, using band pass filter of 0.5 – 30Hz for EEG and 0.15 – 15Hz for EOG. We filtered EMG with a 10Hz high pass.

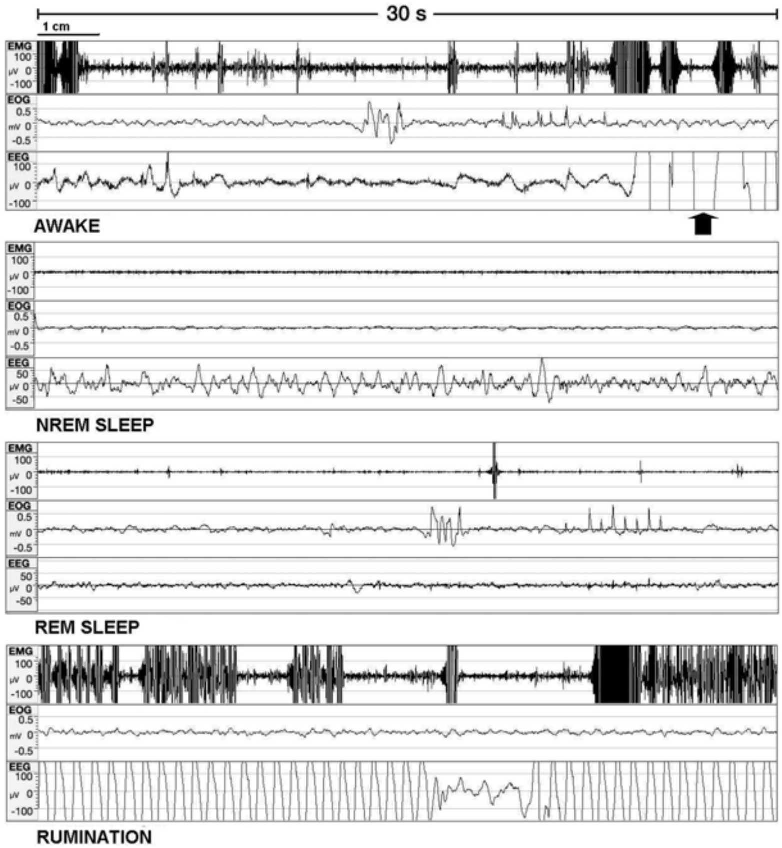
We identified three mutually-exclusive sleep-wake states as awake, NREM sleep and REM sleep on the basis of standard visual analysis and Power spectrum analyses and the following routine criteria developed from human sleep medicine (*Carskadon & Rechtschafen* 2005);

We scored calf to be (Figure 4):

- *awake,* when the EEG was characterized by high frequency, variable amplitude brain electric activity and the EMG displayed clear muscle activity.
- *NREM sleep,* when the EEG showed low frequency, high amplitude activity and EMG amplitude displayed a reduced muscle tone. Occasional rapid eye movements could be seen in EOG during NREM sleep.
- *REM sleep,* when the EEG showed low frequency variable amplitude activity resembling that in Awake combined with low amplitude (0.5 – 5mv) neck muscle EMG, occasional muscle twitches in EMG and typical patterns with rapid eye movements in the EOG.

The observed waveforms constituted three distinct and consistent types, which corresponded well with the criteria for human vigilance states presented by other researchers.

Figure 4. Typical examples of calves electroencephalography (EEG, bottom trace), electro-oculography (EOG, middle trace) and electromyography (EMG, top trace) while awake and during NREM and REM sleep and rumination in three-month-old calves. Band-pass filter of 0.5 – 30Hz was used for EEG and 0.15 – 15 Hz for EOG. EMG was filtered with 10 Hz high pass.



The data epoch length is 30s, band-pass filtered at 0.5 – 30Hz, with recording speed of 1.0 cm/s. Black arrow marks the movement artefact in awake-EEG.

In addition we scored *rumination* when EEG showed a typical prominent rhythmic, low frequency high-amplitude 2-5 Hz artefact covered all brain signals. (Figure 4). Also other head, jaw, ear and eye movements generated movement artefacts in the electrophysiological signals. However, these short-lasting changes did not prevent adequate recognition of the state of vigilance during the 30-s analysis epoch. Most of these occurred when the animal was awake. If the artefacts did not cover the whole of the 30-s epoch, then we scored the epoch according to which vigilance state predominated. However, we scored awake, if the whole 30-s epoch was obscured by artefacts not related to rumination, and the epoch was either followed or preceded by an epoch of awake, since these behaviours occurred nearly always when the animal was awake. If more than ten 30-s epochs were completely obscured by artefacts, and these were not followed by awake state, we then excluded the whole period from the analysis. Usually this was due to detached electrodes and we had to finish the whole recording session

Ruckebush (*Ruckebush* 1972) has stated that adults, such as cows, of many domestic herbivorous species, are spending a great part of their day in a state of a quiet wakefulness called "drowsing", which is different than alert wakefulness. However, his description for this state is not very explicit; electrophysiologically, he described that drowsing is as a mixture of high and low frequencies, during which state cows are resting quiet, possibly ruminating, and their eye lids are relaxed. We could not distinguish a clear drowsing state in calves. If it existed, it might have been hidden under the rumination-caused artefact.

For the sleep phase analyses, if the break between two consecutive vigilance states exceeded 30s, the new bout was defined to begin.

8. SUMMARY OF THE MEASURES USED

Table 4. The summary of places and timing of work, number of animals, treatments, housing and the behavioural and physiological measured used

Paper	**I**
Experiment	A
Place and year of work	Viikki Experiment Farm, University of Helsinki, 1996 – 1999
Number, age and housing of animals	80 calves, 1-12 weeks of age[1], housed in groups of four or individually
Number and description of treatments	Outside yard and heated shelter (n=6) compared to outside yard and unheated shelter unheated shelter (n=6) or similar structure inside the barn (n=5); groups of four inside (n=5) vs. individually housed calves (n=12)
Measured used	Direct observation at 2 min intervals, 12 h/wk; 4-6 times/8-12 wk ; mean age 2,4,7, and 13 wk, Daily health check , weekly weighing

[1] individually housed calves were followed during 1-8 weeks of age

Paper	**II**
Experiment	B
Place and year of work:	Dairy and Swine Research and Development Centre, Agri-Food Canada, 1998
Number, age and housing of animals	48 calves, 1-20 weeks of age kept individually or in pairs.
Number and description of treatments	Concrete-floor individual pens (n=12) compared to concrete-floor double-pens (n[2]=12) or rubber matt individual pens (n=12).
Measured used:	Continuous 24h-recordings 8 times/20 wk; mean age 2,4,6,7,14, and 21 wk, daily health check; bi-weekly weighing.

[2] 24 calves were housed in pairs and mean of the pair-housed calves was a statistical unit

Paper	**III**
Experiment	B
Place and year of work:	Dairy and Swine Research and Development Centre, Agri-Food Canada; year 1998
Number, age and housing of animals	24 calves, 11 -12 weeks of age, kept individually
Number and description of treatments	Concrete-floor individual pens (n=12) compared to rubber matt individual pens (n=12); Before and after relocation
Measured used:	48 hr blood sampling for GH and cortisol secretion at 20 min intervals.

Paper	**IV**
Experiment	C
Place and year of work	Research and education barn, Animal Hygiene, University of Helsinki; year 2000
Number, age and housing of animals	6 calves, 12 – 13 weeks of age, housed in pairs
Number and description of treatments	Electrophysiological recording vs. sleeping behaviour
Measured used	Continuous direct observation for 20 hours/calf with simultaneous polygraphic electrophysiological recordings; daily health check, weekly weighing

9. STATISTICAL ANALYSIS

Here I summarises the statistical methods used in studies I-IV. For more detailed results, the reader may refer to the original papers included at the end of the thesis.

We considered the group of calves or a calf pair a statistical unit and thus we converted data on them to means before statistical analysis. We carried out statistical analyses using SPSS (I, II, and V) or SAS (III, IV) statistical packages.

We studied the effect of age and housing on resting behavior with repeated measures ANOVA (I), with mixed model (II) or applied nonparametric statistical methods with Bonfenronni correction (I). Before the statistical analyses of the data from the experiment II, the total duration and frequency of spent resting on side were log-transformed in order to obtain approximately normal distribution. We used split-plot mixed models for studying the effect of floor type, time of day, and relocation on GH and cortisol secretion (III). Before the statistical analyses, we ln-transformed the concentrations of cortisol and GH in order to obtain approximately normal distribution of errors.

For the analyses of temperature effect, we converted the temperature data to weekly means, and tested with linear regression models (I). General linear mixed model was used for study the relationship between sleeping behavior and EEG findings (IV). In addition, the best behavioural predictors for each vigilance state were then cross-tabulated against the electrophysiological data to see how well each of the behaviours corresponded to a particular vigilance states and where the possible misclassifications occurred. We calculated sensitivity (the percentage of EEG-intervals the behavioural estimates were able to predict to occur) and specificity (the percentage of EEG-intervals the behavioural estimates were able to predict not to occur) for the best models.

And further, Cohen's kappa ($\varkappa$) was used for measuring the agreement between the electrophysiological and behavioural measures of vigilance states and the most common artefact, rumination (IV). If the $\varkappa$ –values is less than 0.2 it indicates poor agreement, 0.2-0.4 fair, 0.41-0.59 moderate, and 0.60-0.79 good, and from 0.80 to 1 very good agreement between ratings (*Landis & Koch* 1977).

We used the matched-pairs t-tests to test for differences in total sleeping times and times spent in different vigilance states between EEG and behavioural sleep recordings. The matched-pair t-test was used also to test the differences in transitions between vigilance states, the p-values were then corrected for the repeated comparisons with the Bonfenronni -correction.

V RESULTS

The most important results appear in this section, which summarises the findings from studies I-IV. For more detailed results, the reader may refer to the original papers included at the end of the thesis.

1. HOW WELL CAN WE IDENTIFY CALVES' SLEEP FROM THEIR RESTING BEHAVIOR (IV)?

1.1 NON-INVASIVE EEG- REGISTERING METHOD IN CALVES

We obtained successful recordings of EEG, EOG, and EMG (i.e. polygraphic recording) from all calves when they were free to move around the pen and interact with their companions. Alltogether 120h of polygraphic data were recorded. Of this 90h 30min was readable data and were selected for visual analyses (15h 4min ± 2h 36 min per calf). The unreadable data mainly resulted from the electrodes becoming detached The calves did not attempt to destroy or manipulate their companions‘ harnesses or other equipment.

1.2 PREDICTING STATES OF VIGILANCE FROM BEHAVIOR.

The best behaviours for predicting sleep (epochs of either REM sleep or NREM sleep) included two behaviours: "resting with head lifted up still" and "resting with neck relaxed" (Table 5). With this combination of behaviours we were able to identify 78 ± 2 % of the epochs of sleep and 82 ± 3 % of those cases in which we had scored the calves as not sleeping with the EEG. Five experimental calves' kappa-value ($\varkappa$) indicated also moderate or good agreement ($\varkappa$; 0.63, 0.71, 0.55, 0.45 and 0.59, respectively, $p<0.001$ for all).

The overall mean durations for REM and NREM states and total sleeping time did not differ when scored with EEG or using the behavioural sleep parameters (Table 6).

Table 5. Results of the general linear mixed models for behavioural predictors for awake and asleep in five 3-month old calves as based on electrophysiological findings.

EEG state		Behavior	B	S.E. (B)	Exp. (B)	p
Awake	1	Eyes open	0.49	0.06	1.64	0.001
	2	Resting head lifted up, head moving actively	2.03	0.13	7.60	0.001
		Standing	0.41	0.07	1.50	0.001
	3	eyes open	0.33	0.07	1.40	0.001
		Resting head lifted up, head moving actively	1.90	0.14	6.68	0.001
		Standing	0.38	0.07	1.46	0.001
Asleep = REM + NREM	1	Resting head lifted still or resting neck relaxed, not supporting the head	0.69	0.09	0.06	0.001

REM = rapid-eye-movement –sleep, NREM = non-rapid-eye-movement-sleep

Table 6. Comparison between the calves' overall mean durations for REM and NREM states and total sleeping time when measured electrophysiologically or using behavioural estimates for the 3-month-old calves` sleep.

Mean (± se) daily duration calves spent in each of the vigilance states	**Mean (± se) percent of time**		**P**
	EEG	**BEHAVIOR**[1]	
NREM	14.0 ± 1.2	13.1 ± 1.1	0.57
REM	11.2 ± 1.6	13.4 ± 1.4	0.11
Total sleep time	25.2 ± 2.3	26.5± 1.0	0.52

[1] Behavioural sleep is defined as calves either resting head lifted up and still (behavioural NREM) or calves resting neck relaxed (behavioural REM).

Table 7. Results of the general linear mixed models for behavioural predictors for different sleep states in five 3-month old calves as based on electrophysiological findings. Only the most relevant results are presented.

EEG state		Behavior	B	S.E. (B)	Exp. (B)	p
NREM	1	Resting head lifted still	0.49	0.09	1.64	0.001
	2	Resting head lifted still	0.49	0.09	1.64	0.001
		eyes closed	0.23	0.08	1.26	0.005
	3	eyes closed	0.27	0.08	1.31	0.001
REM	1	Resting neck relaxed, not supporting the head	0.90	0.11	2.46	0.001
	2	Resting neck relaxed, not supporting the head	0.88	0.11	2.40	0.001
		Rapid eye movements	0.19	0.13	1.21	0.13
	3	eyes closed	0.24	0.09	1.27	0.01
	4	eyes closed	0.83	0.11	2.29	0.02
		Resting neck relaxed, not supporting the head	0.28	0.13	1.33	0.001
		Rapid eye movements	0.21	0.09	1.23	0.03

REM = rapid-eye-movement –sleep, NREM = non-rapid-eye-movement-sleep

We were able to predict awake epochs with two models within 80–81% specificity and sensitivity. Both models included two behaviours: "standing" and "resting head lifted up and moving". If we included the behaviour "eyes open" into the model, we observed only minor effects on the sensitivity and specificity; sensitivity increased from 79.58% to 81% and specificity decreased from 81.4% to 80.06%, respectively. The combination of the two behaviours "standing" and "resting head lifted up and moving" identified 81 ±4 % (range 67 – 92%) of the epochs the calves were awake and 80 ± 3% (range 71-88 %) of those the calves were not awake in the EEG. Kappa values showed moderate or strong agreement ($\varkappa$; 0.66, 0.67, 0.51, 0.50, and 0.60, respectively, $p<0.001$ for all). With this model, however, we still misidentified 18 ± 4% (range 6-25%) of all calves' REM sleep episodes and 17 ± 3% (range 10 – 26%) of all NREM sleep episodes as awake.

If we included only the behavior “eyes open” for the model predicting awake epochs, the model sensitivity was high (92.22%), but specificity was rather poor (42.43%). Nearly half of the calves’ episodes for NREM sleep (43 ± 7%, range 18 – 62%) and REM sleep (7 ± 5%, range 34 – 59%) occurred when the calves’ eyes were scored as open. Kappa values showed fair or moderate agreement (ϰ; 0.35, 0.37, 0.44, 0.40, and 0.21, respectively, p<0.001 for all).

The best model for predicting NREM sleep included the behaviour ‘resting head lifted up still (see adjacent photo). Even though the sensitivity (53.76 %) was not very high, the prediction was very specific (89.36%). The behaviour was able to identify correctly 55 ± 4 % (range 45 % - 66%) of all epochs when the calves were in NREM sleep and 89 ± 2% (84 % - 94%) % when they scored otherwise. Calves’ ϰ-values indicated fair or moderate agreement (ϰ; 0.37, 0.54, 0.45, 0.37 and 0.48, respectively, p<0.001 for all). Furthermore, this model incorrectly predicted 18 ± 4 % (range 5 – 31%) of all REM sleep periods and 9 ± 2 % (range 4- 13%) of all awake periods as NREM sleep.

The best model for predicting the occurrence of REM sleep included the behaviour ‘resting with neck relaxed” (see adjacent photo). This model had poor sensitivity (49.13 %) but it was able to predict very specifically to REM – sleep epochs in the model (90.22%). The behavioural REM estimate was able to identify correctly 61 ± 3 % (range 51 % - 70%) of all epochs when the calves were in REM sleep and 89 ± 2% (83 - 92%) when they were not. Calves’ ϰ-values showed fair, and moderate agreement (ϰ; 0.36, 0.52, 0.48, 0.31 and 0.55, p<0.001 for all). This model incorrectly identified 24 ± 4% (range 14 – 38%) of all NREM sleep periods and 8 ± 2 % (range 6 – 13%) of all Awake as REM.

The behavioural state of “rhythmic chewing while not eating” was very specific and sensitive predictor for the occurrence of rumination (as judged on the basis of electrophysiological data). Observations of this behaviour successfully identified 96 ± 1% (range 93 - 97%) of the periods scored as “ruminating” with the electrophysiological recordings and 94 ± 2 % (range 87

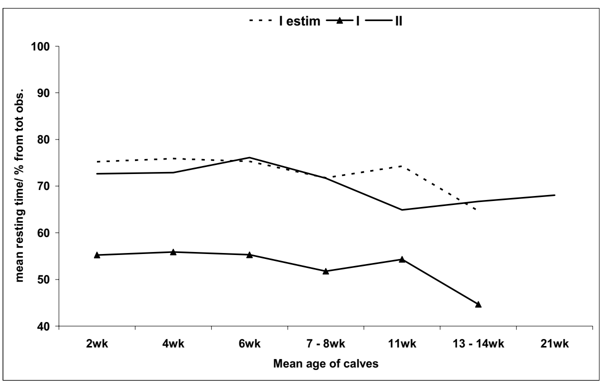
– 96%) of the periods not scored as ruminating. Individual calves' ϰ-values showed a good or a very good agreement between EEG and rumination scores, (ϰ; 0.75, 0.94, 0.86, 0.93 and 0.93, $p<0.001$ for all).

2. How do we describe calves' resting behavior at different ages? (I,II)

2.1 The effect of age on total daily time spent resting and resting in different body postures (I, II)

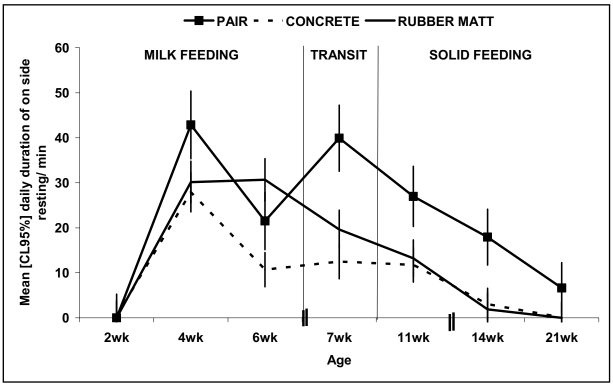
We found significant overall effects of age on total resting time and the daily amount and frequency of different resting body postures (Table 7. at the end of this chapter). Calves rested at least 50% of their day during both of the experiments throughout the ages of 13-21 weeks of ages. In experiment I, we took no observations during night-time hours, but if calves were estimated to rest 80 % of their night hours (from 24 – 06) during that experiment, the calves' mean resting times during experiments I and II were both around 70 % – 80 % of the day (Graph 1).

Graph 1. The effects of aging on calves' mean daily resting time in experiments I and II. The dotted line describes the estimated resting time in experiment I, if calves are estimated to 80% of their night time hours (24 – 06).



The duration of calves' mean resting bouts decreased rapidly between 2 and 4 weeks of age and again slowed between 6 weeks and 14 weeks of age, after which it increased again (II, Graph 2).

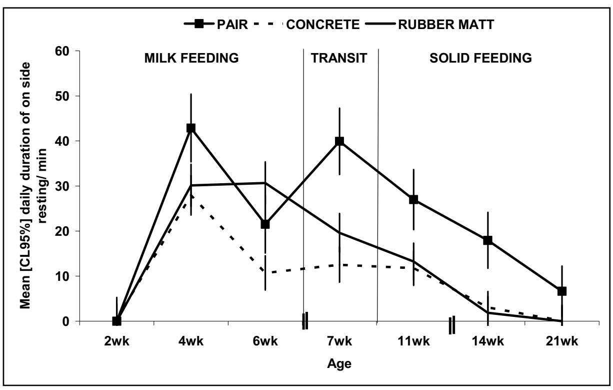
Graph 2. The effect of age on calves' mean daily resting bout duration.



PAIR =Calves housed in pairs in concrete-floor pens, CONCRETE= Calves housed individually in concrete floor pens, RUBBER MAT=calves housed individually in pens with rubber mat floors

Calves rested mostly on the sternum, and thus total sternal rest followed the overall time spent resting. Age affected on-side resting in experiment II, but not in experiment I: calves were not seen to rest on their sides before four weeks of age. The total daily duration spent resting on side decreased, almost disappearing by 21 weeks of age (II, Graph 3.)

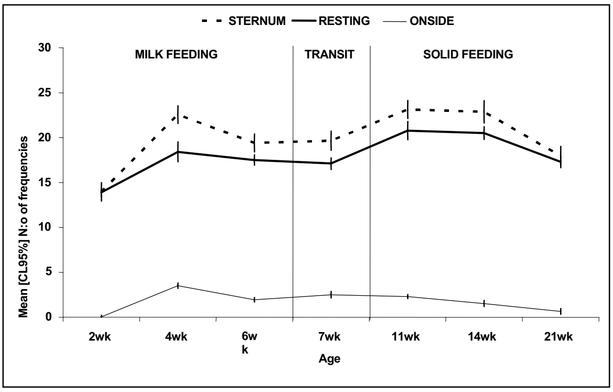
Graph 3. The effect of age on calves' daily time spent resting on side.



PAIR =Calves housed in pairs in concrete-floor pens, CONCRETE= Calves housed individually in concrete floor pens, RUBBER MAT=calves housed individually in pens with rubber mat floors

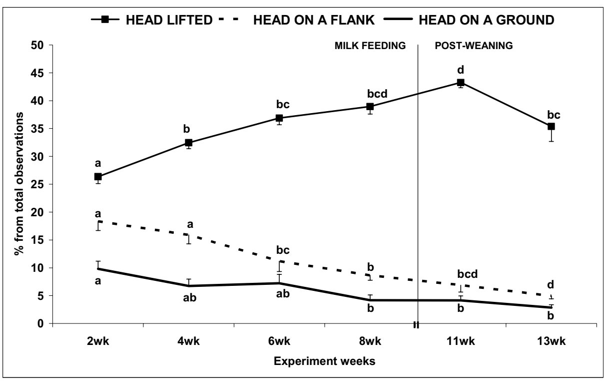
The bout frequency of resting on the sternum increased slightly as the calves aged. In contrast, bouts of resting on the side were infrequent, and remained so as the calves aged (II, Graph 4).

Graph 4. The effect of age on the mean frequency of the calves' bouts of resting, resting on sternum, or resting on side



The time spent resting with the head on the flank and on the ground as decreased while the time spent resting with the head lifted increased from 2 week of age to 11 weeks of age and then dropped slightly at 13 weeks of age (I, Graph 5.).

Graph 5. The effect of age on calves' head postures while resting (I)

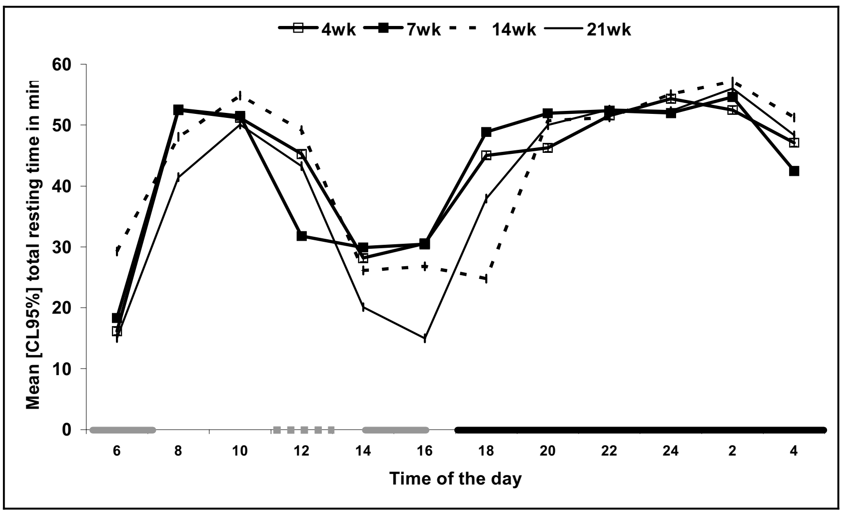


2.2 DAILY RESTING PATTERN AND AGE (II)

Feeding strongly affected the calves' daily resting rhythm: most rest occurred at nighttime or immediately after the morning meal (II, Graph 6).

We saw an interaction between the time of day and the calves' age for the total daily duration of rest (p=0.001); the calves on the transition diet (at 7 weeks of age) tended to be more active at around two hours before and after the extra transition feeding time, when calves received extra concentrate. In addition, older calves (from 14 weeks of age on) were more active during the afternoon feeding (II, Graph 6).

Graph 6. Mean daily duration of total rest at each 2-hour period of the day and at each age. Time of the day * age p=0.001



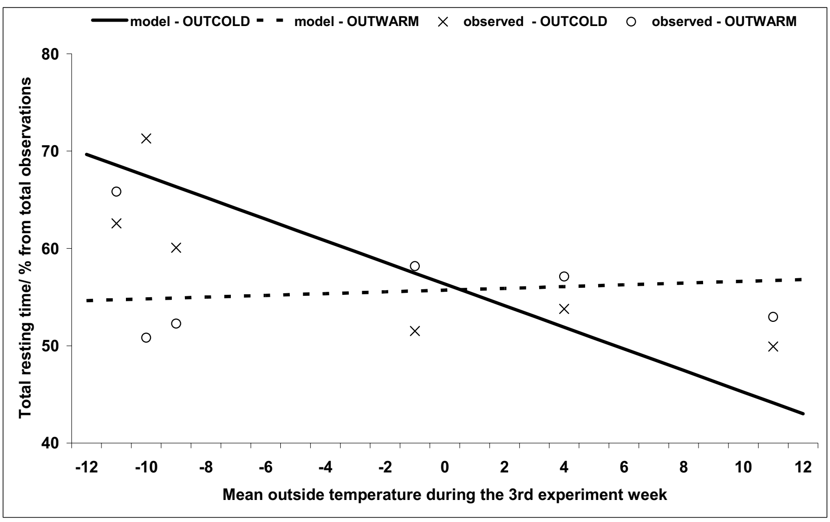
Black bar marks the dark period with lights off, solid grey bars routine feeding times, and dashed grey bars represent the extra feeding time during the transition period.

3. How does the resting behavior change in different environments (I,II)?

3.1 Temperature (I)

During most of the 12-week experiment, the effect of OUTWARM or OUTCOLD (i.e. calves kept outside in the heated or uninsulated shelter) on behavior was independent of the mean, minimum, and maximum temperatures (TEMP, MIN, or MAX); the interaction term was insignificant ($p>0.05$). However, in the temperature range of our experiment, we did observe some interactions; during the third experiment week the time spent resting increased in the OUTCOLD with the decreasing TEMP or MAX, and had no effect on OUTWARM ($p<0.05$, Graph 7.). Also, at the same time the time spent resting with head on the ground increased in the OUTCOLD with the decreasing TEMP, and decreased in the OUTWARM ($p<0.05$).

Graph 7. An estimation of the time the one-month old calves spent resting weekly in the temperature range of experiment I. Dotted and solid lines represent the modelled resting times and crosses and open circles the observed ones.



y=-31+(33*HOUSING)+(0.09*T)+(1.5*WEIGHT)+(HOUSING*T*1.2), HOUSING; housing calves with access to an outside yard and either heated (OUTWARM) or unheated (OUTCOLD) shelters, WEIGHT; the mean weight of a calf-group at the beginning of the 12 -week experiment and T; the mean outside temperature during experiment week.

During the 12-week experiment, the INGROUP calves spent less time resting with the head on the flank than either the OUTCOLD and OUTWARM calves (7.34 ± 0.35% vs. 12.54 ± 1.59% and 12.47 ± 1.0%, respectively, p=0.02 for both). Also the time spent resting with the neck relaxed was significantly less for the INGROUP calves than for the OUTCOLD and OUTWARM calves (12.38 ± 1. 25% vs. 18.24 ± 0.72% and 18.99 ± 1.44%, p=0.01 for both). We found no differences in resting behavior between OUTWARM and OUTCOLD calves and found no differences between INGROUP, OUTGROUP or OUTWARM calves in the amount of total resting and resting on the side, or distance from the shelter wall, or from the neighbouring calves. INGROUP calves also tended to use the shelter less than did the OUTCOLD calves (p=0.07).

3.2 Floor type (II)

We found no statistically significant differences in any of the resting behavior parameters between calves kept on concrete or on rubber mats. There was however, an interaction between housing and the mean duration of resting bouts (p=0.001) calves on rubber mats rested for longer bouts at two weeks of age than did calves on concrete floors (Graph 2, p. 40).

3.3 Social company (I,II)

Social company did not affect the total daily duration of resting time or the duration of resting bouts. There was, however, an interaction between housing and the duration of resting bouts in the experiment II: pair-housed calves from 14 to 21 weeks of age rested for shorter bouts than did individually housed calves of similar age (Graph 2, p. 40).

Pair-housed calves (II) rested on their sides for daily longer and more often than did individually-housed calves over the entire 20-week experiment. Also, during experiment I, two-week-old individually-housed calves rested less on their sides than did group-housed calves (1.4 ± 0.41% vs. 2.46 ± 0.67%, respectively, p=0.03). In addition, over the entire seven-week milk-feeding period, individually-housed calves rested significantly less with their heads on the ground than did calves in groups of four (I: 1.5 ± 0.4% vs. 5.7 ± 1.2%, p<0.05)).

4. HOW WILL STRESS AFFECT THE SECRETION OF REST - RELATED HORMONES SUCH AS GH AND CORTISOL? (III)

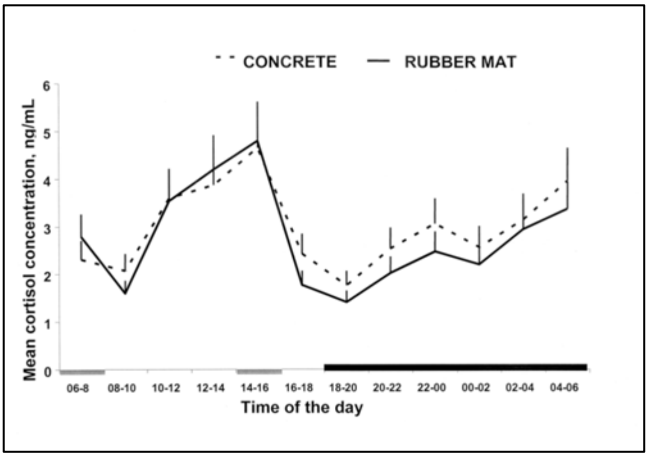
Overall, we detected 4.6 ± 0.6 GH pulses and 5.8 ± 0.8 cortisol pulses /24 h, respectively. Plasma cortisol and GH concentrations were affected by the time of day ($P < 0.001$ for both, Figures 8 and 9). Plasma cortisol concentration peaked twice during the day: at 12:00-14:00 and 4:00-6:00, during or just prior to the feeding times. The respective troughs occurred at 8:00-10:00 and 18:00-20:00, just after the feeding times (Figure 87). Plasma GH concentration was higher during the day time than at night time, and four peaks occurred at intervals of about 6 h (Figure 9).

4.1 FLOOR TYPE

The floor type affected neither the mean GH or cortisol concentrations nor the number of respective pulses. However, we found interactions between the type of floor and the time of day for both the GH and cortisol concentrations ($p=0.001$, for both): calves kept on concrete exhibited higher cortisol concentrations from 16:00 to 24:00, but calves on rubber mats had higher cortisol concentrations between 6:00 and 8:00 (Graph 8). The mean GH concentration was higher for calves on concrete floors than for those on rubber mats at 6:00-8:00 and 18:00-20:00 (Graph 9.).

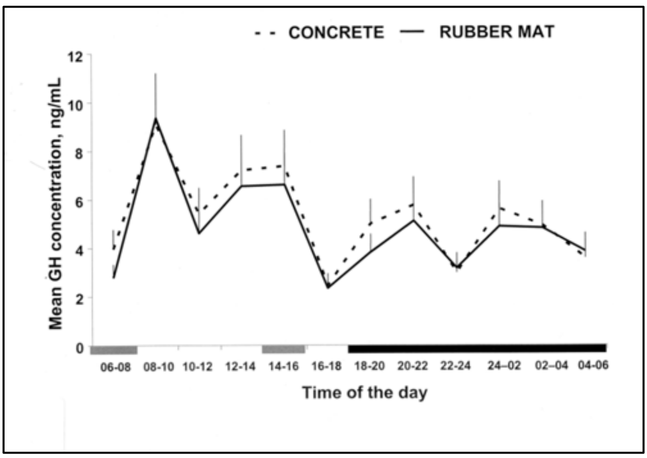
Mean pulsatile measures of cortisol were unaffected by floor type. We found that floor type only slightly affected the pulsatile variables of GH: amplitude tended to be higher for calves on concrete floor than for calves on rubber mats (16.9 ± 3.7 ng/ml vs. 13.3 ± 2.8 ng/ml) and the pulse interval, longer (272 ± 46 min vs. 225 ± 35 min). Floor type did not affect GH troughs.

Graph 8. Overall mean (se) plasma concentrations of cortisol at each time of the day for calves housed individually on concrete (n=12) and rubber mats (n=12).



At the base of the figure black horizontal bars indicate light: dark cycle and grey horizontal bars show the feeding times.

Graph 9. Overall mean (se) plasma concentrations of GH at each time of the day for calves housed individually on concrete (n=12) and rubber mats (n=12).



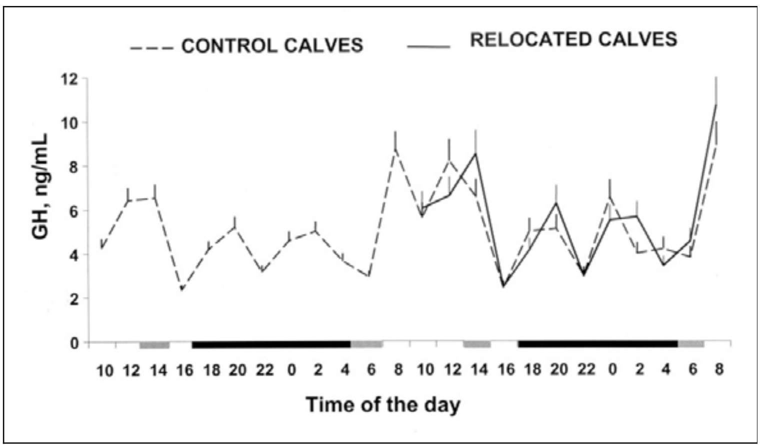
At the base of the figure black horizontal bars indicate light: dark cycle and grey horizontal bars show the feeding times.

4.2. THE EFFECT OF RELOCATION

Plasma concentrations of cortisol or GH were unaffected by the relocation: the mean daily plasma cortisol concentrations both before and after the relocation were similar 2.7 ± 0.4 ng/ml, respectively. We found an interaction between the sampling day, relocation, and the time of the day for GH concentrations (p=0.02) but not for cortisol concentration: Relocation slightly increased the GH concentrations during the four hours after relocation, around mid-night and in the morning (Graph 10).

We found an interaction between the relocation and the sampling day for the pulse interval of cortisol (p=0.02). For control calves, the pulse interval was shorter on the relocation day, than on the to the baseline day (166 ± 30 min vs. 195 ± 29 min), whereas we found the opposite for the relocated calves (216 ± 44 min vs. 181 ± 35 min). In addition, we found an interaction between the relocation and the sampling day for GH pulse variables (p=0.04). Relocated calves had a higher trough after the relocation than did control calves (2.9 ± 0.4 ng/ml vs. 2.4 ± 0.5 ng/ml).

Graph 10. Overall mean (se) GH concentrations of relocated and control calves during base line day and after the relocation at each time of the day for calves



n= 24 for the control calves during the baseline day and n= 12 for the control calves during the relocation day). At the base of the figure black horizontal bars indicate light: dark cycle and grey horizontal bars show the feeding times.

5. SUMMARY OF THE RESULTS

Table 8. The overall main effects of the potential rest-related environmental stressors affecting three-month-old calves' pulsatile cortisol secretions in experiment II

Pulsatile variable	Possible variables affecting cortisol secretion			
	Hardness of flooring	**Relocation**	**Time of the day**	**[1]Interval**
N:o	↔	↔		
Concentration, ng/ml	↔	↔	var.	
Pulse Interval, min	↔	↔	var.	
Trough, ng/ml	↔	↔	var.	↔
Amplitude, ng/ml	↔	↔	var.	↓

N:o= Number of pulses, ↑= increasing, ↓ = decreasing, ↔ = no effect, var. = the effect varies during the time period, [1]Interval= the time between two consecutive pulses

Table 9. The overall main effects of the potential rest-related environmental stressors affecting three-month-old calves' pulsatile GH secretions in experiment II

Pulsatile variable	Possible variables affecting GH secretion			
	Hardness of flooring	**Relocation**	**Time of the day**	**Interval[1]**
N:o	↔	↔		
Concentration, ng/ml	↔	↔	var.	
Pulse Interval, min	↔	↔	var.	
Trough, ng/ml	↔	↑	var.	var.
Amplitude, ng/ml	↑	↔	var.	var.

N:o= Number of pulses,↑= increasing, ↓ = decreasing, ↔ = no effect, var. = the effect varies during the time period, [1]Interval= the time between two consecutive pulses

Table 10. The overall effect of environmental factors on calves' resting behavior. Summary from studies I and II. In study I, only the mean weekly duration was recorded.

Variables affecting resting behavior		Aging I, II	Decreasing temperature I	Social company I, II	Hardness of flooring II	Time of the day II
Total resting time	**Dur**	↓	↑	↔	↔	var.
	Freq	↑		↔	↔	var.
	Bout	var.		↔	↔	var.
Resting on brisket	**Dur**	↑	↑	↔	↔	var.
	Freq	↓		↔	↔	var.
	Bout	↑		↔	↔	var.
Resting on side	**Dur**	↓	↓	↑	↔	var.
	Freq	↑		↑	↔	var.
	Bout	↔		↑	↔	var.
Resting, head lifted	**Dur**	↑	↓			
Shelter usage	**Dur**	↔	↑			
Resting near calf	**Dur**	↔	↔			

Dur = Duration, Freq = Frequency, Bout = Bout length, ↑= increasing, ↓ = decreasing, ↔ = no effect, var. = the effect varies during the time period

VI DISCUSSION

We found that the calves' resting body postures can successfully be used to estimate calves' total sleeping time, and daily duration spent in REM and NREM states, though less successfully to estimate time spent in the different phases of sleep in shorter epochs. This may prove for future research work, for unlike what we had hypothesized a priori, we found, that calves are able to maintain their total daily resting duration, resting bout length, and daily resting rhythm against possible environmental stressors such as hard flooring, absence of social company or relocation.

Also, we found no strong effects either of flooring type or of relocation on plasma concentrations of cortisol or GH. It is possible, that for our lighter calves, the small amount of wood shavings used as bedding have been sufficient to alleviate the discomfort of the hard concrete floor. That relocation had no effect may have reflected the gentle way in which we moved the calves and the similarity of their new environment to their familiar environment and a familiar neighbouring calf in the new room. However, there were some effects of these treatments upon the pulsatile variables that we measured.

Regular blood sampling is needed, though, to quantify these changes, as they are subtle and are evinced mostly in pulse height and circadian rhythms rather than in mean plasma hormone concentrations.

1. HOW WELL CAN WE IDENTIFY CALVES' SLEEP FROM THEIR RESTING BEHAVIOR (IV)?

Electrophysiological data can be recorded non-invasively from freely moving, group-housed calves. We found that the calves' resting body postures are as reliable measure as EEG for calves' total sleeping time, and daily duration spent in REM and NREM states as well as, though less successfully to estimate time spent in the different phases of sleep in shorter epochs.

1.1 NON-INVASIVE EEG - TECHNIQUES

We successfully recorded good quality EEG from the freely-moving group-housed animals. The calves did not attempt to remove or manipulate the harnesses holding the EEG recording equipment on their companions. The optimal method of attaching the electrodes still needs to be determined as some of the electrodes recording eye-movements or neck-muscle activity did not stay in place. This occurred in about 25% of the recorded data. Because, young calves are playful, and head contact occurs often during their playing and fighting bouts, this can affect electrode attachment. In addition, in this experiment, the calves were not dehorned, and growing horns covered with the rubber net seemed to cause some itching. This itching led to head-rubbing, which could compromise the strength of the electrode attachments. Even though only one channel would be necessary to score sleep state (*Takeuchi et al.* 1998), we recommend polygraphic recording due to difficulties with electrode attachments.

The calves were able to adopt their normal resting postures while wearing the harnesses. All the parameters of resting behavior recorded in this study were in line with our previous findings on the normal resting behavior of three-month old calves housed in similar circumstances (study I). Our recording method did not prevent species-specific lying positions, as the calves were observed resting with the head lifted for 11.5 hours, and with the head resting on the ground or on the calf's body for 2.5 hours.

The EEG signal we obtained from calves, was relatively similar to that obtained from adult cows (*Ruckebusch* 1965; *Ruckebusch* 1974b). During the awake phase, the EEG was characterized by the mixture of high-frequency (5 – 25 Hz), variable-amplitude electric brain activity similar to that in adult cattle (Ruckebusch 1965). Low frequency (2-5 Hz) activity occurring in the midst of the fast frequencies was attributed to eye and muscle artifacts. During NREM sleep, the EEG amplitude decreased to less than 100 μV, which corresponds to the findings of Takeuchi et al. (1998), but was less than that found in adult cattle (Ruckebusch 1965). These differences are due to more superficial electrode placement in ours, and Takeuchi's studies (1998) as Ruckebush used implanted electrodes with adult cows (*Ruckebusch* 1965; *Ruckebush* 1972; *Ruckebusch et al.* 1974; *Ruckebusch* 1974b; *Ruckebush* 1975). EMG signal frequency and amplitude decreased to 20Hz and 1-5mV, respectively, indicating a reduction in muscle tone as in adult cattle (*Ruckebusch* 1974b). During REM sleep, the EMG of the neck muscles of our calves was of low amplitude (0.5-5 mV), as in adult cows (*Ruckebusch* 1965; *Ruckebusch* 1974b). We observed occasional muscle twitches, and EOG showed typical patterns with rapid eye movements as in adult cattle (*Ruckebusch* 1965).

Rumination caused a prominent and disturbing artefact in the signal which may have resulted in the misclassification of some NREM sleep since rumination has been shown to occur during NREM sleep but not during REM sleep (*Ruckebusch et al.* 1974).

1.2 EEG AND SLEEP POSTURES

Our results showed that it was possible to make some predictions as to the sleep state of the animals from observations of their behavior. We showed that it is possible to identify calves' sleep if they are resting with their head still or on the flank: according to this study, the occurrence of these behaviors accurately identifies 78% of the time when the calf was asleep. If only eye closure was used to estimate calves' state of sleep, it over estimated the amount of time spent awake, as calves sometimes slept with their eyes half open. This is a type of sleep registered also in adult cattle (*Merrick & Scharp* 1971). In our experiment, the calves' eyes were scored as open if any part of the eye bulb was visible. If only the eyes were used to score sleep, half of the sleep would be miscoded as awake. On the other hand, relying only on body and head postures provides a reasonably good estimate of total sleeping time and total time spent in REM and NREM sleep in calves. Most behavioral studies rely on video-recording nowadays, and with the current video-quality, eyes are not easily seen.

We did, however, find some errors when we predicted calves' sleep states in 30s epochs. Error was lowest in predicting whether the calf was awake or asleep, but our attempts to identify the phase of sleep (REM or NREM sleep) were less successful. A little over 60% of the REM epochs were successfully identified from observations of resting with the neck relaxed. However, only half of the periods of NREM sleep were identified by observations of resting with the head up and still. We explain this poor predictability as a result of frequent transitions between sleep phases, which did not necessarily reflect posture changes in calves. In addition, calves sometimes woke up briefly after REM or NREM sleep bouts without changing their resting postures, thus resulting miscoded awake periods.

2. HOW TO DESCRIBE AND DEFINE CALVES' RESTING BEHAVIOR AT DIFFERENT AGES (I,II) ?

Calves total resting time remains rather constant as the calves aged (papers I, II). After the first weeks of life, total resting time remained around 60 - 80%, depending on the observation interval (I, II). This is very near the 12-13 h hours of resting time that adult cows are ready to defend (*Jensen et al.* 2005). After weaning off milk, the total time spent resting decreased for a while, probably indicating an increased in the time spent eating (II), as has Hepola et al. (2006) shown. Also, previous findings showed slight decreases in rest time during the first three months of age (*Sato & Kuroda* 1993). In our study, the duration of resting bouts increased until 21 weeks of age (II), possibly due to the increased speed of feed intake, and a more developed rumination.

With age, the amount of time spent resting on the sternum increased (I, II) and the proportion of time spent resting on the side decreased (II). The decreasing resting on the side (II) could be partly explained by the reduced pen space available as the calves grew, a finding that is in accordance with those of other studies (*de Wilt* 1985; *Ketelaar-de et al.* 1990; *Le-Neindre* 1993) which have shown that calves spend less time in resting postures that require more space as they grow. Furthermore, in experiment I, we observed no decrease in time spent resting on the side, because the calves had a more spacious resting area (3 m^2 calf in experiment I, compared to 1.9 m^2 in experiment II).

3. HOW AND WHY DOES THE RESTING BEHAVIOR CHANGE IN DIFFERENT ENVIRONMENTS (I, II)?

Calves' total resting time and daily resting rhythm was affected very little by potential environmental stressors, such as low temperature, hard flooring or absence of social company. Calves' circadian resting rhythms were only slightly affected by the weaning; the time spent resting around feeding times decreased after the calves' were weaned off milk.

Low temperatures can directly affect the resting behavior of the un-weaned calves during the first months of their lives, thus increasing their total resting time and decreasing the time spent resting on their sides. Pair-housed calves were able to use the larger pen area and rested more often and for longer durations on their sides than did the individually-housed calves. The softness of the floor had little effect on the behavior of the calves, although the bedding used may have alleviated the hardness of concrete floor.

3.1 Daily resting pattern (II)

Several authors have suggested that sleep rhythms (*Ruckebush* 1975) or activity rhythms (*Ruckebush* 1975; *Veissier et al.* 1989; *Scheibe et al.* 1999) could serve as a measure for the adaptation of animals to their environment. We found no evidence that housing young calves in pairs or individually or on hard concrete floors affected their circadian resting patterns. However, we observed only resting behavior and may have missed changes in their sleep, as calves sleep for only 20-30% of the day, but rest for 60-80% (I, II and IV).

Nor were calves' daily resting patterns disturbed by floor type (II). Feeding and lightning schedules are one of the main regulators of the internal pacemaker (*Buijs et al.* 2003). The lightning schedule remained unchanged during the study, though, the feeding schedule changed as the calves grew, and thus affected calves' resting rhythm; the calves rested mainly at night throughout the experiment. Resting periods were interrupted by feeding times, as Bokkers and Koene (2001) reported. The nocturnal resting time began to decrease 1.5 hours before the morning feeding time (II).

3.2 Floor type (II)

We hypothesized that young calves would rest less on the uncomfortable concrete floor, as previous studies have shown that adult cows rested longer and lay down more frequently on softer floors (*Haley et al.* 2001). We also hypothesized that since calves will likely lose more body heat when lying on their sides, the calves would lie on their sides more on the rubber floor than on the concrete floor. Young calves have very little body insulation (*Gonzalez-Jimenez & Blaxter* 1962), and we observed in experiment I that individually-housed calves on fully-slatted floors rest less on the side during their first weeks of life. We therefore expected that a relation between floor type and resting body postures might emerge. Calves on rubber matts were expected to rest more on the side at a very young, cold-vulnerable age.

However, we found no support for these hypotheses about calves kept individually on concrete or rubber matt floors (II). Perhaps a softer floor is less important to young, and lighter animals, or perhaps the small quantity of bedding sufficiently softened the floor. It is also possible that the rubber mats were not soft enough to make a difference to the light animals. Moreover, because we conducted our experiment during the hot summer time, the room temperature may occasionally risen to over +30 C^0, and the calves may have preferred the cooler, if less bedded, concrete floor. Furthermore, the small differences in the pulsatile secretion of GH and cortisol in study III suggest that different the floor types we used in our study did not strongly affect the calves. We did, however, find subtle changes in pulsatile secretion, suggesting that it might have affected the calves' sleep.

3.3 Temperature (I)

We hypothesized that calves kept in a cold loose-housing environment would adapt to the fluctuating and low temperatures by changing their body postures and total resting time. Schrama et al. (1993; 1995) showed that calves produce more heat when they are standing, so in cool environments, resting is a method of saving energy. We saw some differences in the mean total resting time between the group of calves housed inside the barn and those housed outside, but only during the milk-feeding period. The calves housed outside with an un-heated shelter rested less when the temperature was low (I). Also, the calves outside, both in the heated and in the unheated shelters, rested less on the side, spent more time resting with the head on the flank, and with the neck relaxed than did the group housed calves inside the barn.

Our findings are comparable to those of other studies which have shown that rest increases with the low temperatures in steers (*Gonyou et al.* 1979; *Redbo et al.* 1996), cows (*Malechek & Smith* 1976), and calves by Kauppinen (2000), though Bøe and Havrevoll (1993) found no such trend. Furthermore, Gonzalez-Jimenez and Blaxter (1962), Brunsvold et al. (1985), and de Wilt (1985) have shown that calves in the cold environment rest with their legs curled under the body to conserve energy. However, Bøe and Havrevoll (1993) found no such correlation between the resting postures and low temperature.

While calves tend to seek out a comfortable microclimate in cold temperatures (*Brunsvold et al.* 1985), we found only limited evidence of this in our study : calves housed outside tended to use the shelter more than did calves housed inside, but calves used a heated shelter no more than an unheated one. Interestingly, calves housed inside the barn also used the shelter a great deal, perhaps because they preferred the thick straw bedding or because they sought cover for safe resting. When inside the shelter, the animals mainly chose to stay close to the shelter wall or close to another calf. However, we saw no huddling behavior in low temperatures, as (*Boe* 1990) has shown in calves, and (*Redbo et al.* 1996) in steers. The reason is probably that the covered yard and the straw-bedded shelters gave some cover from wind and rain.

Interestingly, we found that very young calves (under four weeks of age) hardly ever rested on their side. This is in agreement with our expectation based on the possible thermoregulatory function of lying on the side. The younger the calf, the more susceptible it is to cold stress (*Gonzalez-Jimenez & Blaxter* 1962) and would thus be expected to rest less on the side, where heat loss would be greater. The very young calves may have avoided resting on their side in order to conserve heat.

In experiment I, we found that in low temperatures the calves spent more time resting with the neck relaxed. Temperature regulation is impaired during REM-sleep; a rebound effect due to a REM-deprivation cannot be ruled out as an explanation (*Franken et al.* 1993; *Heller* 2005). Temperatures near the lower critical temperature reduce REM-sleep as well as total sleeping time, and during the recovery phase, a rebound effect could occur (*Heller* 2005).

3.4 Social company (i,ii)

Housing young calves on concrete in pairs or individually had no overall effect on the total time they spent resting which is similar to finding of others (*Warnick et al.* 1977; *Albright et al.* 1991; *Chua et al.* 2002). However, de Wilt (1985), and Le Neindre (1993), found that housing calves in groups decreased their resting time, especially if the space was restricted.

However, in both experiments I and II, social company increased the amount of resting on the side. In addition, individually-housed calves in experiment I, spent less time resting with the head on the ground than did the group-housed calves inside the barn.

We could not conclude that pair-housing in the 2.1 x 1.8 m^2 pens (II) disturbed calves' rest comparing to individually housed calves. Resting on the side takes up the most space, and so is likely to be the first resting position to be disturbed by the other calf. Although the area per calf remained constant, the total amount of space in the double pens was larger; this may have made it easier for the calves to lie on their sides. The individually-housed calves may have been unable to fully rest on the side along the short (1.05 m) side of the pen and could use only the long side (1.8m) of the pen. However, the pair-housed calves were able to rest along either side (1.8 * 2.1 m) of a double pen. Also the individually-housed calves in our experiments could easily see neighboring calves, so resting would not have been affected by the calves' attempts to maintain social contact.

In the experiment I, the effect of individual housing on resting postures was unlikely the results of spatial restriction in the individual pen, because both resting on the side and resting with the head on the floor, occurred more often as the calves aged.

4. HOW WILL RELOCATION OR HARD-FLOORING AFFECT THE SECRETION OF GH AND CORTISOL? (III)

Flooring or relocation had no overall effect on the number of pulses, mean GH or cortisol concentrations. However, the effect of time of the day depended on the floor type; calves on concrete floors had higher cortisol concentrations than did those on rubber mats, especially during the night. Relocation had no effect on mean GH or cortisol concentrations and only a small effect on the nature of the pulse variables and the ultradian GH concentration rhythm in plasma. Pulse intervals for cortisol tended to be longer for relocated calves, but the mean cortisol concentrations immediately after the relocation were substantially less than the usual cortisol concentration following acute stress.

To measure environmental effects on hormones, such as cortisol or GH, secreted in a pulsatile manner, requires frequent sampling over large periods of time frames (24 h) to detect episodic changes, as otherwise useful information may be hidden, or results misunderstood because of ultradian, and individual variation. A non-parametrical method previously developed by Woolliams et al. (1993) and Breier et. al (1986) is a practical tool for analyzing differences between repeated blood samplings and to gain additional information on the physiological, ultradian or circadian feed-back mechanisms involved in the blood concentration pattern.

4.1 FLOOR TYPE (III)

The type of flooring affected both cortisol and GH concentrations, but the magnitude of the effects was small (III). These effects varied according to the time of day. Cortisol concentrations were higher at night time for the calves on concrete floors than for the calves kept on rubber mats. The GH concentration was higher throughout the day for calves kept on concrete floors than for those calves kept on rubber mats. During the experiment, the room temperature varied from 16 °C to 26 °C. During the cooler night time, concrete may have been too cold for the calves, whereas during the heat of the day the calves may have found cooler concrete floor preferable to the rubber mats.

Hard flooring has been shown to disturb the rest of adult cows (*Haley et al.* 2001), which may affect their GH and cortisol secretion (*Munksgaard & Løvendahl* 1993). It is possible that for our lighter calves, the small amount of wood shavings used as bedding may have been sufficient to alleviate the discomfort of the hard concrete floor. In steers, the HPA axes have adapted to hard flooring after the first months of housing (Ladewig and Smidt, 1989). Also, as we show here, calves were able to maintain their resting time (II), and thus effects on their GH- or HPA-axes may be small.

In agreement with the minimal effects on mean plasma concentrations of the hormones, floor type did not affect pulsatile cortisol measures and only slightly affected GH pulses. GH pulses tended to be higher and to occur at longer intervals on concrete than on rubber mats. Since cortisol secretion has been shown to increase GH pulse height and the interval between two consecutive GH pulses in rats (*Wehrenberg et al.* 1989), the changes we noted in calves' GH pulses on concrete floors may reflect greater nighttime cortisol concentrations. Whether these small changes were sufficient to cause any biological effects remains unclear.

4.2 Relocation (III)

Relocation had no effect on mean GH or cortisol concentrations, and only slightly affected the nature of the pulse variables. The pulse intervals for cortisol tended to be longer for relocated calves, but the mean cortisol concentrations immediately after the relocation varied between 2 and 6 ng/ml, which is substantially less than the usual cortisol concentration following acute stress caused by handling, castration, or dehorning (*Lay, Jr. et al.* 1992; *Wohlt et al.* 1994; *Fisher et al.* 1996). Thus we found no evidence that this type of relocation is stressful for calves, which is supported by other findings in calves (*Veissier et al.* 2001). That relocation had no effect may have reflected the gentle way in which we moved the calves and the similarity of their new environment to their familiar environment and a familiar neighbouring calf in the new room.

VII CONCLUSION

1. How well can we identify calves' sleep from their resting behavior?

We found that the calves' resting body postures can successfully be used to estimate calves' total sleeping time, and daily duration spent in REM and NREM states, though less successfully to estimate time spent in the different phases of sleep in shorter epochs. Observations of the calf resting quiet with its head up predicted only 55 % of the epochs of NREM sleep. The best behavioural predictor of REM sleep was the calf resting with neck relaxed, which predicted 61 % of the epochs of REM sleep. These behavioural sleep indicators are promising tools, because we have shown here that resting behavior is very stable in calves.

2. How do we describe calves' resting behavior at different ages?

Calves total resting time remains rather constant as the calves aged. However, we observed proportional changes in their resting postures; relaxed resting postures, such as resting on the side or resting with the neck relaxed or both are replaced with resting on a brisket and with head lifted up. This may be due to several things: calves may reduce the exposed surface area of their bodies, when they are young and vulnerable to low temperatures and draft. Also, as older calves rest less with their neck relaxed, it may be a product of reduced REM sleep duration.

3. How does the resting behavior change in different environments?

Calves' resting time is not easily affected by the flooring type, temperatures, or of social company. Nor could we see any effect on the mean duration of their daily resting bouts or resting frequency. Calves were adjusting to lower temperatures by reducing the exposed surface area of their body. Though harder, perhaps the bedded floors were comfortable enough for calves to lie down.

We did measure more frequent and longer bouts of resting on the side in pair-housed calves, though the meaning of these remains to be studied.

Because calves are maintaining their long daily resting time very constant, the resting time may not be sufficiently sensitive to measure environmental quality for young calves, unless the environment is very stressful.

4. How will hard floor type or relocation affect the secretion of GH and cortisol?

We observed only slight changes in the calves' cortisol and GH pulsatile pattern on different floor types. These changes were apparent in the pulse height, and thus required frequent blood sampling. Whether these changes resulted from changes in sleep remains to be studied.

5. Future studies

The following questions require future studies:

- Why do calves rest as much as they do? Other question arising from this is; which environmental factors may disturb calves' rest?

- What are the normal reference values for cattle sleep? Other questions arising from this are; how does sleep develop during aging in calves? Which environmental factors may disturb calves' sleep?

- Are growth hormone and cortisol secretion related to sleep also in cattle? Other questions arising from this are; what do subtle changes in pulsatile cortisol and GH secretion mean for calves? Which environmental factors may disturb calves' GH secretion? Are sleeping-problems affecting the performance of calves?

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"There is a time for many words, and there is also a time for sleep."
Homer: The Odyssey